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10/532,681	02/20/2007	Sergei Anatolievich Lukyanov	U 015745-9	6233

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EXAMINER	
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1632	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

nyuspatactions@ladas.com

Office Action Summary	Application No.	Applicant(s)	
	10/532,681	LUKYANOV ET AL.	
	Examiner	Art Unit	
	WU-CHENG Winston SHEN	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *05/14/2009*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,5-11,13-17 and 27-33* is/are pending in the application.

4a) Of the above claim(s) *9-11 and 14-16* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,5-8,13,17 and 27-33* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *26 April 2005* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *05/14/2009*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant's claim amendments filed on 05/14/2009 have been entered. Sequencing listing filed on 08/31/2009 has been received and entered.

Claims 2-4, 12 18-26, are cancelled. Claims 31-33 are newly added. Claims 6, 13, and 28-30 are amended.

Claims 1, 5-11, 13-17, and 27-33 are pending.

Claims 9-11 and 14-16 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected invention, there being no allowable generic or linking claim.

Claims 1, 5-8, 13, 17, and 27-33 are currently under examination to the extent of elected SEQ ID NO: 9 (705 nucleotides) that encodes the elected SEQ ID No. 10 (234 amino acid residues).

This application 10/532,681 is a 371 of PCT/RU03/00474 filed on 11/05/2003 which claims benefit of 60/425,570 filed on 11/12/2002, and claims benefit of 60/429,795 filed on 11/27/2002, and claims benefit of 60/464,258 filed on 04/21/2003, and claims benefit of 60/480,080 filed on 06/20/2003.

Priority

The following statements was documented in the Non-Final office action mailed on 02/11/2009 and has been updated in response to claim amendments filed on 05/14/2009.

It is noted that provisional applications 60/429,795 filed on 11/27/2002, 60/464,258 filed on 04/21/2003, and 60/480,080 filed on 06/20/2003, did not disclose either SEQ ID No: 10 or

SEQ ID No: 9. The provisional application 60/425,570 filed on 11/12/2002 discloses SEQ ID No 2 that is identical to the SEQ ID No: 10 of instant application, but 60/425,570 filed on 11/12/2002 did not disclose SEQ ID No.9 of instant application since SEQ ID No. 1 and SEQ ID No. 3 disclosed in 60/425,570 are not the same as SEQ IN No. 9 of instant application

Therefore, the priority date of claim 1, which recites SEQ ID No. 10 and its dependent claims 5-8, 13, 17, 27, 28, 29, 31, and 33 is determined to be 11/12/2002, the filing date of provisional application 60/425,570. The priority date of claims 30 and 32, which recites SEQ ID No. 9, is determined to be 11/05/2003, the filing date of PCT/RU03/00474.

In the reply filed on 05/14/2009, Applicant argues that claim 30 should be entitled to the priority date of 11/12/2002. However, Applicant fails to specifically point to the disclosure of SEQ ID No: 9 in the provisional application 60/425,570, filed on 11/12/2002. The priority date of claims 30 and 32, which recites SEQ ID No. 9, remains to be 11/05/2003, the filing date of PCT/RU03/00474.

Sequence compliance

(i) In response to the office action mailed on 02/11/2009 indicating that "The alignment of the sequences listed in Figure 1 requires a sequence identifier", the following amendments to the specification was filed on 05/14/2009.

Figure 1 shows the alignment of GFP (SEQ ID NO: 23), phiYFP (SEQ ID NO: 2), hydrlGFP (SEQ ID NO: 12) and hm2CP (SEQ ID NO: 14) amino acid sequences. Introduced gaps are shown by dots. Residues identical to the corresponding amino acids in GFP are represented by dashes.

(ii) In response to notice of non-compliance mailed on 07/27/2009 regarding proper format for submission of sequences, Applicant filed sequence listing on 08/31/2009. However, the submission by Applicant on 08/31/2009 remains non-compliant, see statements and directions below.

The applicant responded electronically on 8/31/09, but submitted only the "paper copy" of the Sequence Listing; it's included in the "4FL-IR5570-Exchange-08312009-153710.pdf " file, and is in IFW under "SEQLIST".

It is noted that the contents of the "paper" copy of the sequence listing: the applicant will have to make a correction in Sequence 19. The Sequence 19 "<223>" response explaining "<213> Artificial Sequence" is incomplete: it states "phiFYP M1-C1 mutant, derived from the humanized version of the". Please complete "humanized version of the (what)"? Also, no page numbers are allowed in the computer readable form of the sequence listing.

If Applicant wishes to file the computer readable form electronically, he/she must upload it as a separate document, in text format (with a ".txt" file extension), with the document code of "SEQ.TXT". If the applicant has any questions regarding submission format, he/she can contact the Electronic Business Center at 1-866-217-9197 or Anne-Marie Corrigan at 571-272-2501.

Claim Objection

1. Previous objection of claims 1, 28 and 30 for being drawn to a non-elected invention is ***withdrawn*** because the claims have been amended.

Amended claim 1 filed on 05/14/2009 reads as follows: An isolated nucleic acid molecule encoding a fluorescent protein, wherein said protein has at least 85% identity with full length SEQ ID NO: 10.

Amended claim 28 filed on 05/14/2009 reads as follows: The nucleic acid molecule according to the claim 1 which encodes SEQ ID NO: 10.

Amended claim 30 filed on 05/14/2009 reads as follows: The nucleic acid molecule according to the claim 1, having a nucleotide sequence comprising SEQ ID NO: 9.

Claim Rejection - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

2. Previous rejection of claim 29 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention, is ***withdrawn*** because the claim has been amended to be a dependent claim of claim 1.

Claim 29 filed on 05/14/2009 reads as follows: An isolated nucleic acid that hybridizes under stringent conditions to the nucleic acid of claim 1, wherein said nucleic acid encodes a fluorescent protein.

3. Claims 1, 5-8, 13, 17, and 27-33 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which

applicant regards as the invention. *This rejection is necessitated by claim amendments filed on 05/14/2009.*

Claim 13 is unclear because full length SEQ ID NO: 10 is a polypeptide of 234 amino acid residues, which correspond to a 702 nucleotide-long polynucleotide. The limitation "at least 85% identity with full length SEQ ID NO: 10" recited in claim 1 requires at least 596 (702 x 0.85=596.7) identical nucleotide sequences with full length SEQ ID NO: 10. However, claim 13 only requires "identical to a nucleotide sequence of at least 300 nucleotides in length of the nucleic acid molecule according to claim 1", which is further broadening the scope of claim 1 because each nucleic acid molecule encompassed by claim 1 becomes a genus of nucleic acid molecules in claim 13. As a dependent claim of claim 1, the metes and bounds of claim 13 cannot be determined since two distinct scopes are recited.

Claims 31 and 32 are unclear because they are dependent claims of claim 1, and claim 1 recites "at least 85% identity with full length SEQ ID NO: 10" whereas claim 31 recites "at least 90% identity with SEQ ID NO: 10" and claim 32 recites "at least 90% identity with SEQ ID NO: 9". It is noted that in the absence of recitation of "full length SEQ ID NO: 10" in claim 31 and "full length SEQ ID NO: 9" in claim 32, the breadth of claim 31 and 32 encompasses any nucleotide sequence having at least 90% identity with any fragment of SEQ ID NO: 10 or any fragment of SEQ ID NO: 9. Accordingly, claims 31 and 32 recite two distinct scopes and the breadth of claims 31 and 32 is broader than their dependent claim 1 despite of recitation of "at least 90% identity".

With regard to claims 27, 28, and 30, claims 27, 28, and 30 become unclear for similar reasons discussed in the preceding paragraph because "full length SEQ ID N: 10" is recited in

claim 1 whereas claim 27, 28, and 30 recite "SEQ ID NO:10". It is further noted that claim 27 recites the 85% identity which is the same percentage of identity as recited in claim 1. Accordingly, claim 27 recites two distinct scopes and the breadth of claim 27 as written is broader than the scope of claim 1.

With regard to claim 29, claim 29 is further broadening the scope of claim 1 because the nucleic acid molecules hybridize to the nuclei acid of claim 1 encompasses various fragments of nucleic acid sequences encoding SEQ ID No: 10 (a 234-amino-acid-long polypeptide). In other words, each nucleic acid molecule encompassed by claim 1 is broadened to encompass a genus of nucleic acid molecules in claim 29. As a dependent claim of claim 1, the metes and bounds of claim 29 cannot be determined since two distinct scopes are recited.

Claim 33 is unclear because claim 33 depends from claim 1, and claim 1 recites "a fluorescent protein" whereas claim 33 recites "wherein the protein comprises a fluorophore". Applicant is advised to clarify on the record with regard to why claim 33 is further limiting claim 1 in term of the definition of "a florescent protein" recited in claim 1 and the definition of "a fluorophore" recited in claim 33.

Based on discussions provided above, the scope of claim 1 becomes unclear when the scope of claim 1 is narrower than the scope of its dependent claims 13, 27-33. Claims 5-8 and 17 depend from claim 1. Therefore, the metes and bounds of claims 1, 5-8, 13, 17, and 27-33 cannot be determined.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it

pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Written Description

4. Claims 1, 5-8, 13, 17, and 27-30 remain rejected and newly added claims 31-33 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Applicant's arguments filed 05/14/2009 have been fully considered and they are not persuasive. Previous rejection is ***maintained*** for the reasons of record advanced on pages 7-11 of the office action mailed on 02/11/2009.

For the clarity and completeness of this office action, the rejection for the reasons of record advanced on pages 7-11 of the office action mailed on 02/11/2009 is reiterated below with revisions addressing claim amendments filed on 05/14/2009.

Claim 1 amended on 05/14/2009 is directed to an isolated nucleic acid molecule encoding a fluorescent protein, wherein said protein has at least 85% identity with full length SEQ ID NO: 10. Claims 5 and 6 are directed to a vector and an expression vector comprising the nucleic acid of claim 1; Claims 7 and 8 are directed to a cell comprising the nucleic acid of claim 1; Claim 17 is directed to a kit comprising the nucleic acid of claim 1.

Claim 13 is directed to a nucleic acid molecule having a sequence that is substantially the same as, or identical to a nucleotide sequence of at least 300 nucleotides in length of the nucleic acid molecule according to claim 1. As discussed in the rejection under 112 second, claim 13 is further broadening the scope of claim 1.

Claim 29 is directed to an isolated nucleic acid that hybridizes under stringent conditions to the nucleic acid of claim 1, wherein said nucleic acid encodes a fluorescent protein. As discussed in the rejection under 112 second, claim 29 is further broadening the scope of claim 1.

Newly added claim 31 is directed to the nucleic acid molecule according to claim 1, wherein said nucleic acid molecule encodes a fluorescent protein having at least 90% identity with SEQ ID NO: 10. Newly added claim 32 is directed to the nucleic acid molecule according to Claim 1, having a nucleotide sequence having at least 90% identity with SEQ ID NO: 9.

The specification discloses SEQ ID No. 10 (a 234-amino acid long polypeptide) is a humanized version of the phiYFG-M1, which is a mutant form of phiYFP generated by random mutagenesis of phiYFP (an YFP isolated from microorganism *Philalidium* sp.). The specification discloses that SEQ ID No. 9 (a 705-nucleotide long polynucleotide) encodes SEQ ID No. 10. The specification discloses the alignment between GFP (from jelly fish), phiYFP, hydriGFP, and hm2CP in Figure 1. The phiYFP shares only ~50% identity with well characterized GFP (from jelly fish) (See Figure 1 disclosed in specification as well as alignments provided in this office action under 102 rejections below).

Based on sequence search performed by the Examiner, it is noted that SEQ ID No. 10 (phiYFG-M1) shares 96% identity with phiYFP (an YFP isolated from microorganism *Philalidium* sp.), see alignment below.

```
RESULT 1
Q6RYS7_9CNID
ID   Q6RYS7_9CNID            Unreviewed;       234 AA.
AC   Q6RYS7;
DT   05-JUL-2004, integrated into UniProtKB/TrEMBL.
DT   05-JUL-2004, sequence version 1.
DT   24-JUL-2007, entry version 13.
DE   Yellow fluorescent protein.
OS   Phialidium sp. SL-2003.
OC   Eukaryota; Metazoa; Cnidaria; Hydrozoa; Hydroida; Leptomedusae;
OC   Campanulariidae; Phialidium.
OX   NCBI_TaxID=258839;
RN   [1]
RP   NUCLEOTIDE SEQUENCE.
RX   PubMed=14963095; DOI=10.1093/molbev/msh079;
RA   Shagin D.A., Barsova E.V., Yanushevich Y.G., Fradkov A.F.,
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RA   Lukyanov K.A., Labas Y.A., Semenova T.N., Ugalde J.A., Meyers A.,
RA   Nunez J.M., Widder E.A., Lukyanov S.A., Matz M.V.;
RT   "GFP-like proteins as ubiquitous metazoan superfamily: evolution of
RT   functional features and structural complexity.";
RL   Mol. Biol. Evol. 21:841-850(2004).
CC   ---------------------------------------------------------------------
CC   Copyrighted by the UniProt Consortium, see http://www.uniprot.org/terms
CC   Distributed under the Creative Commons Attribution-NoDerivs License
CC   ---------------------------------------------------------------------
DR   EMBL; AY485333; AAR85349.1; -; mRNA.
DR   HSSP; P42212; IB9C.
DR   GO; GO:0008218; P:bioluminescence; IEA:InterPro.
DR   GO; GO:0006091; P:generation of precursor metabolites and energy; IEA:InterPro.
DR   GO; GO:0018298; P:protein-chromophore linkage; IEA:InterPro.
DR   InterPro; IPR011584; GFP_related.
DR   InterPro; IPR000786; Green_fl_protein.
DR   Pfam; PF01353; GFP; 1.
DR   PRINTS; PR01229; GFLUORESCENT.
DR   ProDom; PD013786; Green_fl_protein; 1.
PE   2: Evidence at transcript level;
SQ   SEQUENCE   234 AA;  26051 MW;  0E7FZDRAAE735D9A CRC64;

  Query Match             96.0%;  Score 1231;  DB 2;  Length 234;
  Best Local Similarity   96.6%;  Pred. No. 1.2e-102;
  Matches  226;  Conservative    3;  Mismatches    5;  Indels    0;  Gaps    0;

Qy          1 MSEGALLFHGKIPYVVEMEGNVDGHTFSIRGKGYGDASVGKVDAQFICTTGDVPVPWSTL 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 MSEGALLFHGKIPYVVEMEGNVDGHTFSIRGKGYGDASVGKVDAQFICTTGDVPVPWSTL 60

Qy         61 VTTLTYGAQCFAKYGPELKDFYKSCMPDGYVQERTITFEGDGNFKTRAEVTFENGSVYNR 120
              ||||||||||||||||||||||||||||:|||||||||||||||||||||||||||||||
Db         61 VTTLTYGAQCFAKYGPELKDFYKDCMPDGYVQERTITFEGDGVFKTRAEVTFENGSVYNR 120

Qy        121 VKLNGQGFKKDGHVLGKNLEFNFTPHCLYIWGDQANHGLKSAFKICHITGSKGDFIVAD 180
              |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        121 VKLNGQGFKKDGHVLGKNLEFNFTPHCLYIWGDQANHGLKSAFKIMHEITGSKEDFIVAD 180

Qy        181 HTQMNTPIGGGPVHVPEYHHMSYHVKLSKDVTDHRDNMSLKETVRAVDCRKTYL 234
              ||||||||||||||||||:|::||| ||||||||||||| ||||||||||||
Db        181 HTQMNTPIGGGPVHVPEYHHITTHVTLSKDVTDHRDNMSLVETVRAVDCRKTYL 234
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The specification does not provide any information regarding the structure-function correlation of phiYFP in terms which amino acids are *necessary and sufficient* for phiYFP to be a fluorescent protein. The nucleotide sequences that encodes a fluorescent protein with at least 85% identity with SEQ ID No. 10, variants, and fragments thereof encompassed within the genus of nucleotide molecules encodes 85% fluorescent protein with at least 85% identity with SEQ ID No. 10, have not been disclosed. The specification discloses isolation of polynucleotide SEQ ID No. 9 encoding polypeptide SEQ ID No. 10 by random mutagenesis. There is no evidence on the record of a relationship between the structure of any nucleic acid encoding a fluorescent protein and the claimed nucleic acid molecules encodes a fluorescent protein with at least 85% identity with SEQ ID No. 10, over the entire length of SEQ ID No: 10, that would provide any

reliable information about the structure of other nucleic acid encoding a fluorescent protein within the genus. In the absence of a functional assay it would not be possible to test variants of the claimed sequences for biological activity. Also with regard to the allelic variants encompassed by the claims, the skilled artisan cannot envision the structure of such a variant because such variants are randomly produced in nature, and cannot be predicted from a known sequence. The specification does not teach any characteristics of an "allelic" variant that would distinguish it from a non-natural variant constructed by the hand of man. In view of the above considerations one of skill in the art would not recognize that applicant was in possession of the necessary common features or attributes at sequence level possessed by member of the genus. Consequently, since Applicant was in possession of only the nucleotide sequences SEQ ID No.10 encoded by SEQ ID No. 9 and since the art recognized variation among the species of the genus of nucleic acid molecules encodes a fluorescent protein with at least 85% identity with SEQ ID No. 10, the SEQ ID No. 9 encoding SEQ ID No. 10 was not representative of the claimed genus. This is because the amino acids that are necessary and sufficient for phiYFP to be a fluorescent protein have not been disclosed and SEQ ID SEQ ID No. 9 encoding SEQ ID No. 10 was obtained by random mutagenesis, which does not disclose structure-function relationship. Therefore, Applicant was not in possession of the genus of the nucleotide sequences that encodes a fluorescent protein with at least 85% identity with SEQ ID No. 10 over the entire length of SEQ ID No: 10 as encompassed by the claims.

It is further noted that claim 29 is directed to the limitation "hybridization under stringent conditions". The specification only discloses an example (a species) of various conditions that Applicant regards as "stringent conditions". The art recognizes that "hybridization under

stringent conditions" is determined by variations in multiple factors (detergents, salts, hydrogen bond competitor, and temperatures etc.). The specification discloses examples of stringent conditions (See paragraph [0056], 2007/0298412, publication of instant application) without clear definition what stringent conditions are. Therefore, the genus encompassed by "hybridization under stringent conditions" is not described to render a skilled artisan to possess the sequences by hybridization that encodes a fluorescent protein having at least 85% identity with SEQ ID No. 10. University of California v. Eli Lilly and Co., 43 USPQ2d 1398, 1404, 1405 held that to fulfill the written description requirement, a patent specification must describe an invention and do so in sufficient detail that one skilled in the art can clearly conclude that "the inventor invented the claimed invention."

Applicant's arguments

Applicant argues that the specification teaches the relevance of GFP and Anthozoan protein structure to the structure of the disclosed proteins (p. 1, I. 13-26; Figure 1). The art teaches a well-studied and highly predictable structure of GFP; see, for example, Yang et al. ((1996) Nat. Biotech 14:1246-51) (Exhibit A) and Ormo et al. ((1996) Science 273:1392-95) (Exhibit B). The art teaches that this structure is conserved amongst all fluorescent proteins and can be used to make predictions as to which amino acids can be substituted in these proteins and how without loss of protein function; see, for example, Matz et al. ((1999) Nat Biotech 17:969-973) (Exhibit C). Thus, one of ordinary skill in the art would understand from the specification that the proteins encoded by the claimed nucleic acids should have a structure resembling that of the well-studied and highly predictable structure of GFP while maintaining at least 85% identity with SEQ ID NO: 10. For example, the specification and the art teach the reliance of GFP and Anthozoan proteins upon their fluorophore for their fluorescence character (specification, p. 1, l. 27-31; Matz et al.) (See page 8 of Applicant's arguments filed on 05/14/2009).

Applicant argues that the specification teaches 7 examples of proteins that are encoded by nucleic acids of the claimed genus (SEQ ID NOs: 2, 4, 6, 8, 10, 18 and 20) and 2 examples of proteins from other species (SEQ ID NOs: 12 and 14) that could be aligned with GFP so as to identify all of the residues that should be conserved to maintain fluorescence activity. Indeed, the specification provides teachings of how to perform such alignments; see Figure 1. The Applicants submit that one of ordinary skill in the art would be able to use this alignment in Figure 1 as well as alignments with other GFP-like proteins know in the art to identify the amino acids to be conserved, for example, the amino acids comprising the fluorophore, so as to retain the fluorescence character of the protein. Moreover, such alignments would demonstrate to the artisan that the disclosed proteins share only 12.8% conserved amino acids with GFP and one another (see Exhibit D, which is an alignment of the disclosed proteins to one another and to GFP; asterisks indicate conserved amino acids); accordingly, the artisan would also recognize from such alignments that strict conservation of most amino acids of these proteins is not required to maintain protein function. Thus, the specification provides sufficient written description such that one of ordinary skill in the art would know that a high degree of amino acid substitution could be tolerated by the proteins of this family including the protein encoded by SEQ ID NO: 10 without loss of fluorescence, and would be able to determine which amino acid substitutions those would be (See page 9 of Applicant's arguments filed on 05/14/2009).

Applicant argues that in support of this expectation that a high degree of amino acid substitutions in these proteins can be tolerated without losing protein function, the art teaches a plethora of GFP mutations that preserve GFP fluorescence activity. For example, Heim et al. ((1996) Current Biol. 6:178-182) (Exhibit E) teaches six mutants comprising mutations in 10 residues of GFP (Table 1). Siemering et al. ((1996) Current Biol. 6(12):1653-63) (Exhibit F) teaches seven additional mutants (mgfp4, mgfpB, mgfpA, mgfp5, mgfp4 + Y66H, mgfpA + Y66H) comprising mutations in another three residues. Yang et al. ((1998) J Biol Chem 273(14):8212-8216). (Exhibit G) teaches two additional mutants comprising mutations in an additional two residues. In addition, the art teaches a plethora of other fluorescent proteins having minimal identity with GFP. For example, Wiedenmann et al. ((2000) PNAS 97(26):14091-6) (Exhibit H) teaches three fluorescent proteins of Anemonia sulcata (asFP499, asFP522, asFP595; see Table 1) that, as a group, share only 12.6% identity to GFP (see Figure 5,

"consensus" line). Matz et al. (Exhibit C) teaches six fluorescent proteins from Anthozoa that, as a group, share only 11% identity with GFP (see Figure 1, "cns. All" line), and how the fluorescence activity of GFP and other fluorescent proteins relies upon these conserved residues. Bevis et al. ((2002) Nat. Biotechnol 20(1):83-7) (Exhibit I) teaches 7 mutants of one of these Anthozoan proteins, dsRed, (N42H, N42Q, DsRedl, dsRed2, DsRed.T1, DsRed.T3, DsRed.T4; see p. 83, col. 2, para. 3-4, p. 84, Table 1), all of which retain fluorescent activity. Campbell et al. ((2002) PNAS 99(12):7877-82) (Exhibit J) teaches 4 more mutants of dsRed (1125R, dimer2, tdimer2, mRFP1; see paragraph bridging pages 7878-9, and Table 1) that retain fluorescent activity. Shaner et al. ((2004) Nat Biotechnol 22(12):1567-72) (Exhibit K) teaches a multitude more dsRed-based mutants with improved extinction coefficients, photostability, and a variety of fluorescence spectra (see, for example, Table 1) (See pages 9-10 of Applicant's arguments filed on 05/14/2009).

Response to Applicant's arguments

The Examiner acknowledges that the status of art does provide information pertaining to GFP as cited by Applicant (Exhibits A, B, C, E, F, G, and H) and dsRFP (Exhibits I, J, and K). The Examiner also acknowledges that Exhibit D, which was not disclosed in the specification originally filed on 04/26/2005, Applicant provides alignments between SEQ ID NOs:2, 4, 6, 8, 10, 18 and 20, and 2 examples of proteins from other species (SEQ ID NOs:12 and 14) and demonstrates to the artisan that the disclosed proteins share only 12.8% conserved amino acids with GFP and one another (see Exhibit D, which is an alignment of the disclosed proteins to one another and to GFP; asterisks indicate conserved amino acids).

However, Applicant is reminded that the claimed invention is directed to SEQ ID No: 10, which is neither a GFP nor a dsRFP. In this regard, it is noted that the excitation-emission spectra of phiYFP are distinct from the excitation-emission spectra of GFP and dsRFP, and the identical sequences between GFP, dsRFP and phiYFP are only 12.8%, as Applicant states in the remarks. Accordingly, there is no evidence on the record how an artisan can base on the teachings regarding GFP and dsRFP to determine the structure-function relationship of the genus of sequences of phiYFP claimed by Applicant. It is worth noting that SEQ ID No: 10 is a 234-amino acid long polypeptide and is a humanized version of the phiYFG-M1, which is a mutant

form of phiYFP generated by random mutagenesis of phiYFP (an YFP isolated from microorganism *Philalidium* sp. The mutation was not based on any know structure-function relationship of phiYFP. Therefore, the mere alignment between SEQ ID NOs: 2, 4, 6, 8, 10, 18 and 20, and SEQ ID NOs: 12 and 14 presented in Exhibit D, and their low identity to GFP fails to provide structure-function relationship of the sequences encompassed by 85% identity with full length SEQ ID No: 10 recited in claim 1, and certainly fails to support written description required for further broadened scope recited in claims 13 and 29.

With regard to the limitation "hybridizes under stringent conditions" recited in claim 29, it is worth noting that the specification does not define what conditions are considered as "stringent conditions". The nucleic acid molecules hybridize to the nuclei acid of claim 1 encompasses various fragments of nucleic acid sequences encoding SEQ ID No: 10 (a 234-amino-acid-long polypeptide encoded by SEQ ID No: 9 that is a 705-nucleotide-long polynucleotide). In the absence of clearly defined "stringent conditions", the specification fails to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Furthermore, pertaining to hybridization language, Applicant's attention is directed to Claim 3, Example 6: DNA hybridization on pages 21-23 of the revised Written Description Training Materals on 03/25/2008, with excerpt cited below, available online <http://www.uspto.gov/web/menu/written.pdf>.

"Thus, the claimed genus necessarily includes partial structures of SEQ ID NO: 1. The disclosure of SEQ ID NO: 1 combined with the knowledge in the art regarding hybridization would put one in possession of the genus of nucleic acids that would hybridize under stringent conditions to SEQ ID NO: 1. However, without a recognized correlation between structure and function, those of ordinary skill in the art would not be able to identify without further testing which of those nucleic acids that hybridize to SEQ ID NO: 1 would also encode a polypeptide that binds to NDG receptor and stimulates tyrosine kinase activity. Thus, those of ordinary skill in the art would not consider the applicant to have been in possession of the claimed genus of nucleic acids based on the single species disclosed."

Scope of Enablement

5. Claims 1, 5-8, 13, 17, and 27-30 remain rejected and newly added claims 31-33 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for an isolated nucleic acid molecule comprising of SEQ ID No. 9 that encodes a fluorescent protein consisting of SEQ ID No. 10, and a vector/cell/kit comprising SEQ ID No. 9 that encodes a fluorescent protein consisting of SEQ ID No. 10, **does not** reasonably provide enablement for (1) any isolated nucleic acid molecule encodes a fluorescent protein other than SEQ ID No. 9 that encodes a fluorescent protein consisting of SEQ ID No. 10, or (2) any vector/cell/kit comprising any isolated nucleic acid molecule encodes a fluorescent protein other than SEQ ID No. 9 that encodes a fluorescent protein consisting of SEQ ID No. 10. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims. Applicant's arguments filed 05/14/2009 have been fully considered and they are not persuasive. Previous rejection is ***maintained*** for the reasons of record advanced on pages 11-14 of the office action mailed on 02/11/2009.

For the clarity and completeness of this office action, the rejection for the reasons of record advanced on pages 11-14 of the office action mailed on 02/11/2009 is reiterated below with revisions addressing claim amendments filed on 05/14/2009.

Enablement is considered in view of the Wands factors (MPEP 2164.01(a)). The court in Wands states: "Enablement is not precluded by the necessity for some experimentation such as routine screening. However, experimentation needed to practice the invention must not be undue experimentation. The key word is 'undue,' not 'experimentation.' " (*Wands*, 8 USPQ2d 1404).

Clearly, enablement of a claimed invention cannot be predicated on the basis of quantity of experimentation required to make or use the invention. "Whether undue experimentation is needed is not a single, simple factual determination, but rather is a conclusion reached by weighing many factual considerations." (*Wands*, 8 USPQ2d 1404). The factors to be considered in determining whether undue experimentation is required include: (1) the quantity of experimentation necessary, (2) the amount or direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims. While all of these factors are considered, a sufficient amount for a *prima facie* case is discussed below.

The basis of this scope of enablement is hinged on the lack of enabling support on the structure/function relationship to make and use any isolated nucleic acid molecule comprising nucleotide sequences encoding a fluorescent protein having at least 85% identity with SEQ ID No. 10 recited in independent claim 1, and further broadened scope of nucleic acid molecules encompassed by dependent claims 13 and 29.

The nature of the instant invention is drawn to an isolated nucleic acid molecule comprising nucleotide sequences. Claim 1 amended on 05/14/2009 is directed to an isolated nucleic acid molecule encoding a fluorescent protein, wherein said protein has at least 85% identity with full length SEQ ID NO: 10. Claims 5 and 6 are directed to a vector and an expression vector comprising the nucleic acid of claim 1; Claims 7 and 8 are directed to a cell comprising the nucleic acid of claim 1; Claim 17 is directed to a kit comprising the nucleic acid of claim 1. Claim 13 is directed to a nucleic acid molecule having a sequence that is substantially the same as, or identical to a nucleotide sequence of at least 300 nucleotides in length of the nucleic acid molecule according to claim 1. As discussed in the rejection under

112 second, claim 13 is further broadening the scope of claim 1. Claim 29 is directed to an isolated nucleic acid that hybridizes under stringent conditions to the nucleic acid of claim 1, wherein said nucleic acid encodes a fluorescent protein. As discussed in the rejection under 112 second, claim 29 is further broadening the scope of claim 1.

As discussed in more details in the rejection under 35 U.S.C 112 second, and 35 U.S.C 112 first written description, the breadth of the claims encompasses any isolated nucleic acid molecule encodes a fluorescent protein in addition to SEQ ID No. 9 that encodes a fluorescent protein consisting of SEQ ID No. 10, and any vector/cell/kit comprising any isolated nucleic acid molecule encodes a fluorescent protein in addition to SEQ ID No. 9 encodes a fluorescent protein consisting of SEQ ID No. 10. Regarding claim 13, the limitation "at least 85% identity with full length SEQ ID NO: 10" recited in claim 1 requires at least 596 (702 x 0.85=596.7) identical nucleotide sequences with full length SEQ ID NO: 10. However, claim 13 only requires "identical to a nucleotide sequence of at least 300 nucleotides in length of the nucleic acid molecule according to claim 1", which is further broadening the scope of claim 1 because each nucleic acid molecule encompassed by claim 1 becomes a genus of nucleic acid molecules in claim 13. Regarding claim 29, the claim is further broadening the scope of claim 1 because the nucleic acid molecules hybridize to the nuclei acid of claim 1 encompasses various fragments of nucleic acid sequences encoding SEQ ID No: 10 (a 234-amino-acid-long polypeptide). In other words, each nucleic acid molecule encompassed by claim 1 is broadened to encompass a genus of nucleic acid molecules in claim 29.

The specification discloses SEQ ID No. 10, a 234-amino acid long polypeptide, is a humanized version of the phiYFG-M1, which is a mutant form of phiYFP generated by random

mutagenesis of phiYFP (an YFP isolated from microorganism *Philalidium* sp.). The specification discloses that SEQ ID No. 9 (a 705-nucleotide long polynucleotide) encodes SEQ ID No. 10. The specification discloses the alignment between GFP (from jelly fish), phiYFP, hydriGFP, and hm2CP in Figure 1. The phiYFP shares only about 50% identity with well characterized GFP (from jelly fish) (See Figure 1 disclosed in specification as well as alignments provided in this office action under 102 rejections).

Based on sequence search performed by the Examiner, it is noted that SEQ ID No. 10 (phiYFG-M1) shares 96% identity with phiYFP (an YFP isolated from microorganism *Philalidium* sp.), see alignment in the preceding written description rejection.

The specification does not provide any guidance regarding the structure-function correlation of phiYFP in terms which amino acids are necessary and sufficient for phiYFP to be a fluorescent protein. It would require undue experimentation for an artisan to determine which amino acids are necessary and sufficient for phiYFP-M1 (i.e. the claimed SEQ ID No. 10) to be a fluorescent protein to support the breadth of the claims.

In the art, it is unpredictable how variations of sequences in a given fluorescent protein would affect its function as a fluorescent protein. For instance, **Shagi et al.** teaches that homologs of the green fluorescent protein (GFP), including the recently described GFP-like domains of certain extracellular matrix proteins in Bilaterian organisms, are remarkably similar at the protein structure level, yet they often perform totally unrelated functions, thereby warranting recognition as a superfamily (See Shagin et al., GFP-like proteins as ubiquitous metazoan superfamily: evolution of functional features and structural complexity, *Mol Biol Evol.* 21(5):841-50, 2004).

Furthermore, it is unpredictable regarding connection between a fragment, variant, or a genetic mutation and the functionality of the resulting fragment, variant, or a genetic mutant polypeptide. Considering SEQ ID No: 9, which encodes SEQ IOD No:10, there are 705 nucleotides in SEQ ID No:9 and 10% of non-identical sequence will include 70 nucleotides. Considering only regular nucleotides (A,T G, and C) as possible nucleotides of each position, there will be 4^{70} (1.39×10^{42}) variations of sequences encompassed by the limitation "90% sequence identity with SEQ ID NO: 9" recited in claim 32. In this regard, **Parmley et al.**, 2007 teaches that even silent SNPs (single nucleotide polymorphisms) encoding the same amino acid residues are not necessarily neutral with regard to their effects on the functions of polypeptides, and there are two additional mechanisms affecting the function of a given polypeptide: (1) modification of protein structure and activity, mediated by induction of translational pausing during co-translational protein folding, and (2) modification of protein abundance mediated by alteration in mRNA stability via changed secondary structures of mRNA, which in turn leads to perturbation in protein synthesis (See abstract, Parmley et al., How do synonymous mutations affect fitness? *Bioessays*, 29(6): 515-9, 2007). In other words, alterations in either protein folding or translational efficiency could result in changed protein functions encoded by synonymous mutations.

In view of the state of the art, the unpredictability in the art, and the lack of specific guidance and working examples in the specification, one of skill in the art would have to perform undue experimentation to make and use the claimed invention as recited in claims 1, 5-8, 13, 17, and 27-33.

Applicant's arguments and ***Response to Applicant's arguments***

(i) Applicant argues that, contrary to the Examiner's assertions, and as discussed above, the nucleic acids of the pending claims are limited to those that encode fluorescent proteins having a sequence identity of at least 85% with SEQ ID NO: 10. Accordingly, the claims are not unduly broad (See page 13 of Applicant's remarks filed on 05/14/2009).

In response, the breadth of claim 1 becomes unclear because dependent claims of claim 1 further broaden the scope of claim 1. Claim 13 is directed to a nucleic acid molecule having a sequence that is substantially the same as, or identical to a nucleotide sequence of at least 300 nucleotides in length of the nucleic acid molecule according to claim 1. As discussed in the rejection under 112 second, claim 13 is further broadening the scope of claim 1. Claim 29 is directed to an isolated nucleic acid that hybridizes under stringent conditions to the nucleic acid of claim 1, wherein said nucleic acid encodes a fluorescent protein. As discussed in the rejection under 112 second, claim 29 is further broadening the scope of claim 1.

(ii) Applicant argues that the specification teaches 7 examples of proteins (SEQ ID NOs: 2, 4, 6, 8, 10, 18 and 20) having a sequence identity of at least 85% with SEQ ID NO: 10. Methods of identifying wild type proteins having a sequence identity of at least 85% with a known protein, for example, degenerate PCR and BLAST searching, are well understood in the art, and thus, one of ordinary skill in the art would know how to identify other nucleic acid sequences that encoding wild type fluorescent proteins having a sequence identity of at least 85% with SEQ ID NO: 10 (See page 13 of Applicant's remarks filed on 05/14/2009).

Applicant argues that in view of the art, they have also provided sufficient guidance and written examples to enable the species of nucleic acids encoding mutants of wild type fluorescent proteins encompassed by the claimed genus. The specification teaches the relevance of GFP and Anthozoan protein structure to the structure of the disclosed proteins (p. 1, I. 13-26; Figure 1). Applicant argues that one of ordinary skill in the art would know how to use the alignment in Figure 1 as well as alignments with other GFP-like proteins know in the art to identify the amino acids to be conserved, for example, the amino acids comprising the fluorophore, so as to retain the fluorescence character of the protein. More importantly, such alignments would demonstrate to the artisan that the disclosed proteins share only 12.8% conserved amino acid residues with

GFP and one another (see Exhibit D, which is an alignment of the disclosed proteins to one another and to GFP); accordingly, the artisan would also recognize from such alignments that strict conservation of most amino acids of these proteins is not required to maintain protein function. Thus, the specification provides sufficient guidance and working examples such that one of ordinary skill in the art would know that a high degree of amino acid substitution could be tolerated by the proteins of this family including protein encoded by SEQ ID NO: 10 without loss of fluorescence, and would be able to determine which amino acid substitutions those would be.

Applicant argues that in support of this expectation that a high degree of amino acid substitutions in these proteins can be tolerated without losing protein function, the art teaches a plethora of GFP mutations that preserve GFP fluorescence activity. For example, Heim et al. (Exhibit E) teaches six mutants comprising mutations in 10 residues of GFP (Table 1). Siemering et al. (Exhibit F) teaches seven additional mutants (mgfp4, mgfpB, mgfpA, mgfp5, mgfp4 + Y66H, mgfpA + Y66H) comprising mutations in another three residues. Yang et al. (Exhibit G) teaches two additional mutants comprising mutations in an additional two residues. The art also teaches a plethora of other fluorescent proteins having minimal identity with GFP. For example, Wiedenmann et al. (Exhibit H) teaches three fluorescent proteins of Anemonia sulcata (asFP499, asFP522, asFP595; see Table 1) that, as a group, share only 12.6% identity to GFP (see Figure 5, "consensus" line). Matz et al. (Exhibit C) teaches six fluorescent proteins from Anthozoans that, as a group, share only 11% identity with GFP (see Figure 1, "cns. All" line), and how the fluorescence activity of GFP and other fluorescent proteins relies upon these conserved residues. Bevis et al. (Exhibit I) teaches 7 mutants of one of these Anthozoan proteins, dsRed, (N42H, N42Q, DsRedl, dsRed2, DsRed.T1, DsRed.T3, DsRed.T4; see p. 83, col. 2, para. 3-4, p. 84, Table 1), all of which retain fluorescent activity. Campbell et al. (Exhibit J) teaches 4 more mutants of dsRed (1125R, dimer2, tdimer2, mRFP1; see paragraph bridging pages 7878-9, and Table 1) that retain fluorescent activity. Shaner et al. (Exhibit K) teaches a multitude more dsRed-based mutants with improved extinction coefficients, photostability, and a variety of fluorescence spectra (see, for example, Table 1) (See pages 14-15 and 17 of Applicant's remarks filed on 05/14/2009).

In response, the Examiner acknowledges that the status of art does provide information pertaining to GFP as cited by Applicant (Exhibits A, B, C, E, F, G, and H) and dsRFP (Exhibits I, J, and K). The Examiner also acknowledges that Exhibit D, which was not disclosed in the specification originally filed on 04/26/2005, Applicant provides alignments between SEQ ID NOs:2, 4, 6, 8, 10, 18 and 20, and 2 examples of proteins from other species (SEQ ID NOs:12 and 14) and demonstrates to the artisan that the disclosed proteins share only 12.8% conserved amino acids with GFP and one another (see Exhibit D, which is an alignment of the disclosed proteins to one another and to GFP; asterisks indicate conserved amino acids).

However, Applicant is reminded that the claimed invention is directed to SEQ ID No:10, which is not a GFP nor a dsRFP. In this regard, it is noted that the excitation-emission spectra of phiYFP are distinct from the excitation-emission spectra of GFP and dsRFP, and the identical sequences between GFP, dsRFP and phiYFP are only 12.8%, which Applicant states in the remarks. Accordingly, there is no evidence on the record how an artisan can base on the teachings regarding GFP and dsRFP to determine the structure-function relationship of the genus of sequences of phiYFP claimed by Applicant. It is worth noting that SEQ ID No: 10 is a 234-amino acid long polypeptide and is a humanized version of the phiYFG-M1, which is a mutant form of phiYFP generated by random mutagenesis of phiYFP (an YFP isolated from microorganism *Philalidium* sp. The mutation was not based on any know structure-function relationship of phiYFP. Therefore, the mere alignment between SEQ ID NOs: 2, 4, 6, 8, 10, 18 and 20, and SEQ ID NOs: 12 and 14 presented in Exhibit D, and their low identity to GFP fails to provide structure-function relationship of the sequences to enable the scope encompassed by 85% identity with full length SEQ ID No: 10 recited in claim 1, and fails to enable further broadened scope recited in claims 13 and 29.

With regard to the limitation "hybridizes under stringent conditions" recited in claim 29, it is worth noting that the specification does not define what conditions are considered as "stringent conditions". The nucleic acid molecules hybridize to the nuclei acid of claim 1 encompasses various fragments of nucleic acid sequences encoding SEQ ID No: 10 (a 234-amino-acid-long polypeptide encoded by SEQ ID No: 9 that is a 705-nucleotide-long polynucleotide). In the absence of clearly defined "stringent conditions", the specification fails

to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

(iii) Applicant argues that the pending claims do not recite limitations on protein function other than that the encoded proteins have a fluorescence activity. Accordingly, Shagin et al.'s teachings of how proteins of similar 3D-structures may perform totally unrelated functions from one another are not relevant to the pending claims (See page 16 of Applicant's remarks filed on 05/14/2009).

In response, as documented in the maintained scope of enablement rejection, Shagi et al. teaches that homologs of the green fluorescent protein (GFP), including the recently described GFP-like domains of certain extracellular matrix proteins in Bilaterian organisms, are remarkably similar at the protein structure level, yet they often perform totally unrelated functions, thereby warranting recognition as a superfamily (See Shagin et al., GFP-like proteins as ubiquitous metazoan superfamily: evolution of functional features and structural complexity, *Mol Biol Evol.* 21(5):841-50, 2004). Applicant's arguments that Shagin et al.'s teachings of how proteins of similar 3D-structures may perform totally unrelated functions from one another are not relevant to the pending claims have been fully considered and found not persuasive.

It is worth noting again that the disclosed proteins share only 12.8% conserved amino acids with GFP and one another (see Exhibit D, which is an alignment of the disclosed proteins to one another and to GFP; asterisks indicate conserved amino acids). These 12.8% conserved amino acid residues indicate the potentially necessary amino acid residues for GFP-like domains. However, it is well established in the status of art that the amino acid residues sufficient for functionality of a protein, in this case excitation-emission spectra of a fluorescent protein, needs proper folding into a functional 3D-structures. It is worth noting again that SEQ ID No: 10 is a 234-amino acid long polypeptide and is a humanized version of the phiYFG-M1, which is a mutant form of phiYFP generated by random mutagenesis of phiYFP (an YFP isolated from microorganism *Philalidium* sp. The mutation was not based on any know structure-function relationship of phiYFP. Therefore, it is unpredictable regarding connection between a fragment (encompassed by claims 13 and 29 of instant application), variant, or a genetic mutant genetic

mutation and the functionality of the resulting fragment, variant, or a genetic mutant polypeptide. Considering SEQ ID No: 9, which encodes SEQ IOD No: 10, there are 705 nucleotides in SEQ ID No: 9 and 10% of non-identical sequence will include 70 nucleotides. Considering only regular nucleotides (A, T G, and C) as possible nucleotides of each position, there will be 4^{70} (1.39×10^{42}) variations of sequences encompassed by the limitation "90% sequence identity with SEQ ID NO: 9" recited in claim 32. In this regard, **Parmley et al.**, 2007 teaches that even silent SNPs (single nucleotide polymorphisms) encoding the same amino acid residues are not necessarily neutral with regard to their effects on the functions of polypeptides, and there are two additional mechanisms affecting the function of a given polypeptide: (1) modification of protein structure and activity, mediated by induction of translational pausing during co-translational protein folding, and (2) modification of protein abundance mediated by alteration in mRNA stability via changed secondary structures of mRNA, which in turn leads to perturbation in protein synthesis (See abstract, Parmley et al., How do synonymous mutations affect fitness? *Bioessays*, 29(6): 515-9, 2007). In other words, alterations in either protein folding or translational efficiency could result in changed protein functions encoded by synonymous mutations.

Claim Rejection - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless -

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

(e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

6. Claims 1, 5-8, 13, 17, and 27-30 remain rejected and claims 31-33 are newly rejected under 35 U.S.C. 102(e) as being anticipated by Baubet et al. (Baubet et al., US 2008/0213879, publication date 09/04/2008, Division of US 6,936,475, which is a Continuation of PCT/EP01/07057, WO 2001/092300, filed on 06/01/2001). Applicant's arguments filed 05/14/2009 have been fully considered and they are not persuasive. Previous rejection is ***maintained*** for the reasons of record advanced on pages 14-26 of the office action mailed on 02/11/2009.

For the clarity and completeness of this office action, the rejection for the reasons of record advanced on pages 14-26 of the office action mailed on 02/11/2009, is reiterated below with revisions addressing claim amendments filed on 05/14/2009.

The following claim interpretations are applied in this rejection.

(i) Amended claim 13 filed on 05/14/2009 reads as follows: A nucleic acid molecule having a sequence that is substantially the same as, or identical to a nucleotide sequence of at least 300 nucleotides in length of the nucleic acid molecule according to claim 1. The limitation "at least 300 nucleotides in length of the nucleic acid molecule" reads on those identical nucleotide sequences that are not necessarily continuous. Accordingly, this limitation requires 100 amino acid residues (which correspond to 300 nucleotides) identical to SEQ ID No: 10 (full length 234 amino acid residues). In other words, this limitation requires at least 42.7% (100/234=42.7%) identical to full length SEQ ID No: 10.

(ii) Amended claim 1 filed on 05/14/2009 reads as follows: An isolated nucleic acid molecule encoding a fluorescent protein, wherein said protein has at least 85% identity with full length SEQ ID NO: 10. As discussed in the rejection of claims 1, 5-8, 13, 17, and 27-33 under 35 U.S.C 112 second and claim interpretation stated in **(i)**, the scope of claim 1 becomes unclear when the scope of claim 1 is narrower than the scope of its dependent claims 13 and 27-33.

Accordingly, as the scope of claim 1 becomes unclear, dependent claims 5-8 and 17 become unclear. Therefore, to accommodate the scope of dependent claims of claim 1, the limitation "at least 85% identity with full length SEQ ID No: 10" recited in claim 1 is interpreted to encompass a fluorescent protein having a fragment with at least 9 out 10 amino acid residues identical to the sequences of the full length SEQ ID NO: 10 (i.e. 90% identity recited in newly added claim 31).

With regard to claims 1, 5-8, 13, and 27-33, Baubet et al. teaches a modified bioluminescent system comprising a fluorescent molecule covalently linked with a photoprotein, wherein said link between the two proteins has the function to stabilize the modified bioluminescent system and allowing the transfer of the energy by Chemiluminescence Resonance Energy Transfer (CRET) in a host cell (See abstract and Figures 9-11, Bauet et al. US 2008/0213879). Baubet et al. teaches DNA construct with CMV promoter drive the expression of nucleic acid sequences encoding sequences of mutated GFP, followed by the sequences of Poly A of SV40 (See Figure 1, Baubet et al. US 2008/0213879)

With regard to the limitation "kit" recited in claim 17, Baubet et al. teaches kit for measuring the transfer of energy *in vivo* or *in vitro* contains at least one of the polypeptides according to the invention or the polynucleotide according to the invention and the reagents necessary for visualizing or detecting the said transfer in presence or in absence of a molecule of interest (See paragraph [0027], Baubet et al., US 2008/0213879)

The following sequence alignments are SEQ ID No. 10 and SEQ ID No. 9 of instant application aligned with disclosed SEQ ID numbers by Baubet et al. (Baubet et al., US 2008/0213879).

(A) Alignment of SEQ ID No. 10 of instant application with SEQ ID numbers 1-6 of Baubet et al.

```
RESULT 1
US-11-149-177-1 (SEQ ID No. 1)
; Sequence 1, Application US/11149177
; Publication No. US20080213879A1
; GENERAL INFORMATION
;  APPLICANT: BAUBET, VALERIE
;  APPLICANT:LE MOUELLIC, HERVE
;  APPLICANT:BRULET, PHILIPPE
;  TITLE OF INVENTION: CHIMERIC GFP-AEQUORIN AS BIOLUMINESCENT Ca++ REPORTERS
;  TITLE OF INVENTION:AT THE SINGLE CELL LEVEL
;  FILE REFERENCE: 03495-0207-00000
;  CURRENT APPLICATION NUMBER: US/11/149,177
;  CURRENT FILING DATE: 2005-06-10
;  PRIOR APPLICATION NUMBER: 09863901
;  PRIOR FILING DATE: 2001-05-24
;  PRIOR APPLICATION NUMBER: 60/208,314
;  PRIOR FILING DATE: 2000-06-01
;  PRIOR APPLICATION NUMBER: 60/210,526
;  PRIOR FILING DATE: 2000-06-06
;  PRIOR APPLICATION NUMBER: 60/255,111
;  PRIOR FILING DATE: 2000-12-14
;  NUMBER OF SEQ ID NOS: 48
;  SOFTWARE: PatentIn Ver. 2.1
; SEQ ID NO 1
;  LENGTH: 432
;  TYPE: PRT
;  ORGANISM: Aequorea victoria
US-11-149-177-1

  Query Match             50.5%;  Score 648;  DB 4;  Length 432;
  Best Local Similarity   53.9%;  Pred. No. 6.5e-60;
  Matches  123;  Conservative   40;  Mismatches   61;  Indels    4;  Gaps    2;

Qy          1 MSSGALLFHGKIPYVVEMEGNVDGHTFSIRGKGYGDASVGKVDAQFICTTGDVPVPWSTL 60
              || |  || | :| :||::|:|:|| ||: |:| |||: ||:  :|||||| :|||| ||
Db          1 MSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTL 60

Qy         61 VTTLTYGAQCFAKYGPELK--DFYKSCMPDGYVQERTITFEGDGNFKTRAEVTFENGSVY 118
              ||||||| |||::|   :|  ||:|| ||:|||||||| |: |||:|||||| ||  ::
Db         61 VTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLV 120

Qy        119 NRVKLNGQGFKKDGHVLGKNLEFNFTPHCLYIWGDQANHGLKSAFKICHEITGSKGDFIV 178
              ||::| |  ||:||::||  ||:|:  | :||  |:  :|:|: ||| | |    |   :
Db        121 NRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKANFKIRHNI--EDGSVQL 178

Qy        179 ADHTQMNTPIGGGPVHVPEYHHMSYHVKLSKDVTDHRDNMSLKETVRA 226
              ||| | ||||| ||| :|: |::|    ||||  : ||:| | | | |
Db        179 ADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTA 226


RESULT 2
US-11-149-177-2 (SEQ ID No. 2)
; Sequence 2, Application US/11149177
; Publication No. US20080213879A1
; GENERAL INFORMATION
;  APPLICANT: BAUBET, VALERIE
;  APPLICANT:LE MOUELLIC, HERVE
;  APPLICANT:BRULET, PHILIPPE
;  TITLE OF INVENTION: CHIMERIC GFP-AEQUORIN AS BIOLUMINESCENT Ca++ REPORTERS
;  TITLE OF INVENTION:AT THE SINGLE CELL LEVEL
;  FILE REFERENCE: 03495-0207-00000
;  CURRENT APPLICATION NUMBER: US/11/149,177
;  CURRENT FILING DATE: 2005-06-10
;  PRIOR APPLICATION NUMBER: 09863901
;  PRIOR FILING DATE: 2001-05-24
;  PRIOR APPLICATION NUMBER: 60/208,314
;  PRIOR FILING DATE: 2000-06-01
;  PRIOR APPLICATION NUMBER: 60/210,526
;  PRIOR FILING DATE: 2000-06-06
;  PRIOR APPLICATION NUMBER: 60/255,111
;  PRIOR FILING DATE: 2000-12-14
;  NUMBER OF SEQ ID NOS: 48
;  SOFTWARE: PatentIn Ver. 2.1
; SEQ ID NO 2
;  LENGTH: 441
;  TYPE: PRT
```

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; ORGANISM: Aequorea victoria
US-11-149-177-2

  Query Match             50.5%;  Score 648;  DB 4;  Length 441;
  Best Local Similarity   53.9%;  Pred. No. 6.7e-60;
  Matches  123;  Conservative   40;  Mismatches   61;  Indels    4;  Gaps    2;

Qy          1 MSSGALLFHGKIPYVVEMEGNVDGHTFSIRGKGYGDASVGKVDAQFICTTGDVPVPWSTL 60
              || |  || | :| :||::|:|:|| ||: |:| |||: ||:  :|||||| :|||| ||
Db          1 MSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTL 60

Qy         61 VTTLTYGAQCFAKYGPELK--DFYKSCMPDGYVQERTITFEGDGNFKTRAEVTFENGSVY 118
              ||||||| |||::|   :|  ||:|| ||:|||||||| |: |||:|||||| ||  ::
Db         61 VTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLV 120

Qy        119 NRVKLNGQGFKKDGHVLGKNLEFNFTPHCLYIWGDQANHGLKSAFKICHEITGSKGDFIV 178
              ||::| |  ||:||::||  ||:|:  | :||  |:  :|:|: ||| | |    |   :
Db        121 NRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKANFKIRHNI--EDGSVQL 178

Qy        179 ADHTQMNTPIGGGPVHVPEYHHMSYHVKLSKDVTDHRDNMSLKETVRA 226
              ||| | ||||| ||| :|: |::|    ||||  : ||:| | | | |
Db        179 ADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTA 226


RESULT 3
US-11-149-177-3 (SEQ ID No. 3)
; Sequence 3, Application US/11149177
; Publication No. US20080213879A1
; GENERAL INFORMATION
;  APPLICANT: BAUBET, VALERIE
;  APPLICANT:LE MOUELLIC, HERVE
;  APPLICANT:BRULET, PHILIPPE
;  TITLE OF INVENTION: CHIMERIC GFP-AEQUORIN AS BIOLUMINESCENT Ca++ REPORTERS
;  TITLE OF INVENTION:AT THE SINGLE CELL LEVEL
;  FILE REFERENCE: 03495-0207-00000
;  CURRENT APPLICATION NUMBER: US/11/149,177
;  CURRENT FILING DATE: 2005-06-10
;  PRIOR APPLICATION NUMBER: 09863901
;  PRIOR FILING DATE: 2001-05-24
;  PRIOR APPLICATION NUMBER: 60/208,314
;  PRIOR FILING DATE: 2000-06-01
;  PRIOR APPLICATION NUMBER: 60/210,526
;  PRIOR FILING DATE: 2000-06-06
;  PRIOR APPLICATION NUMBER: 60/255,111
;  PRIOR FILING DATE: 2000-12-14
;  NUMBER OF SEQ ID NOS: 48
;  SOFTWARE: PatentIn Ver. 2.1
; SEQ ID NO 3
;  LENGTH: 450
;  TYPE: PRT
;  ORGANISM: Aequorea victoria
US-11-149-177-3

  Query Match             50.5%;  Score 648;  DB 4;  Length 450;
  Best Local Similarity   53.9%;  Pred. No. 6.9e-60;
  Matches  123;  Conservative   40;  Mismatches   61;  Indels    4;  Gaps    2;

Qy          1 MSSGALLFHGKIPYVVEMEGNVDGHTFSIRGKGYGDASVGKVDAQFICTTGDVPVPWSTL 60
              || |  || | :| :||::|:|:|| ||: |:| |||: ||:  :|||||| :|||| ||
Db          1 MSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTL 60

Qy         61 VTTLTYGAQCFAKYGPELK--DFYKSCMPDGYVQERTITFEGDGNFKTRAEVTFENGSVY 118
              ||||||| |||::|   :|  ||:|| ||:|||||||| |: |||:|||||| ||  ::
Db         61 VTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLV 120

Qy        119 NRVKLNGQGFKKDGHVLGKNLEFNFTPHCLYIWGDQANHGLKSAFKICHEITGSKGDFIV 178
              ||::| |  ||:||::||  ||:|:  | :||  |:  :|:|: ||| | |    |   :
Db        121 NRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKANFKIRHNI--EDGSVQL 178

Qy        179 ADHTQMNTPIGGGPVHVPEYHHMSYHVKLSKDVTDHRDNMSLKETVRA 226
              ||| | ||||| ||| :|: |::|    ||||  : ||:| | | | |
Db        179 ADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTA 226


RESULT 4
US-11-149-177-4 (SEQ ID No. 4)
; Sequence 4, Application US/11149177
; Publication No. US20080213879A1
; GENERAL INFORMATION
;  APPLICANT: BAUBET, VALERIE
;  APPLICANT:LE MOUELLIC, HERVE
;  APPLICANT:BRULET, PHILIPPE
;  TITLE OF INVENTION: CHIMERIC GFP-AEQUORIN AS BIOLUMINESCENT Ca++ REPORTERS
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;  TITLE OF INVENTION:AT THE SINGLE CELL LEVEL
;  FILE REFERENCE: 03495-0207-00000
;  CURRENT APPLICATION NUMBER: US/11/149,177
;  CURRENT FILING DATE: 2005-06-10
;  PRIOR APPLICATION NUMBER: 09863901
;  PRIOR FILING DATE: 2001-05-24
;  PRIOR APPLICATION NUMBER: 60/208,314
;  PRIOR FILING DATE: 2000-06-01
;  PRIOR APPLICATION NUMBER: 60/210,526
;  PRIOR FILING DATE: 2000-06-06
;  PRIOR APPLICATION NUMBER: 60/255,111
;  PRIOR FILING DATE: 2000-12-14
;  NUMBER OF SEQ ID NOS: 48
;  SOFTWARE: PatentIn Ver. 2.1
; SEQ ID NO 4
;  LENGTH: 468
;  TYPE: PRT
;  ORGANISM: Aequorea victoria
US-11-149-177-4

  Query Match             50.5%;  Score 648;  DB 4;  Length 468;
  Best Local Similarity   53.9%;  Pred. No. 7.3e-60;
  Matches  123;  Conservative   40;  Mismatches   61;  Indels    4;  Gaps    2;

Qy          1 MSSGALLFHGKIPYVVEMEGNVDGHTFSIRGKGYGDASVGKVDAQFICTTGDVPVPWSTL 60
              || |  || | :| :||::|:|:|| ||: |:| |||: ||:  :|||||| :|||| ||
Db          1 MSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTL 60

Qy         61 VTTLTYGAQCFAKYGPELK--DFYKSCMPDGYVQERTITFEGDGNFKTRAEVTFENGSVY 118
              ||||||| |||::|   :|  ||:|| ||:|||||||| |: |||:|||||| ||  :: 
Db         61 VTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLV 120

Qy        119 NRVKLNGQGFKKDGHVLGKNLEFNFTPHCLYIWGDQANHGLKSAFKICHEITGSKGDFIV 178
              ||::| |  ||:||::||  ||:|:  | :||  |:  :|:|: ||| | |    |   :
Db        121 NRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKANFKIRHNI--EDGSVQL 178

Qy        179 ADHTQMNTPIGGGPVHVPEYHHMSYHVKLSKDVTDHRDNMSLKETVRA 226
              ||| | ||||| ||| :|: |::|    ||||  : ||:| | | | |
Db        179 ADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTA 226
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RESULT 5

US-11-149-177-5 **(SEQ ID No. 5)**

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; Sequence 5, Application US/11149177
; Publication No. US20080213879A1
; GENERAL INFORMATION
;  APPLICANT: BAUBET, VALERIE
;  APPLICANT:LE MOUELLIC, HERVE
;  APPLICANT:BRULET, PHILIPPE
;  TITLE OF INVENTION: CHIMERIC GFP-AEQUORIN AS BIOLUMINESCENT Ca++ REPORTERS
;  TITLE OF INVENTION:AT THE SINGLE CELL LEVEL
;  FILE REFERENCE: 03495-0207-00000
;  CURRENT APPLICATION NUMBER: US/11/149,177
;  CURRENT FILING DATE: 2005-06-10
;  PRIOR APPLICATION NUMBER: 09863901
;  PRIOR FILING DATE: 2001-05-24
;  PRIOR APPLICATION NUMBER: 60/208,314
;  PRIOR FILING DATE: 2000-06-01
;  PRIOR APPLICATION NUMBER: 60/210,526
;  PRIOR FILING DATE: 2000-06-06
;  PRIOR APPLICATION NUMBER: 60/255,111
;  PRIOR FILING DATE: 2000-12-14
;  NUMBER OF SEQ ID NOS: 48
;  SOFTWARE: PatentIn Ver. 2.1
; SEQ ID NO 5
;  LENGTH: 477
;  TYPE: PRT
;  ORGANISM: Aequorea victoria
US-11-149-177-5

  Query Match             50.5%;  Score 648;  DB 4;  Length 477;
  Best Local Similarity   53.9%;  Pred. No. 7.6e-60;
  Matches  123;  Conservative   40;  Mismatches   61;  Indels    4;  Gaps    2;

Qy          1 MSSGALLFHGKIPYVVEMEGNVDGHTFSIRGKGYGDASVGKVDAQFICTTGDVPVPWSTL 60
              || |  || | :| :||::|:|:|| ||: |:| |||: ||:  :|||||| :|||| ||
Db          1 MSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTL 60

Qy         61 VTTLTYGAQCFAKYGPELK--DFYKSCMPDGYVQERTITFEGDGNFKTRAEVTFENGSVY 118
              ||||||| |||::|   :|  ||:|| ||:|||||||| |: |||:|||||| ||  :: 
Db         61 VTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLV 120

Qy        119 NRVKLNGQGFKKDGHVLGKNLEFNFTPHCLYIWGDQANHGLKSAFKICHEITGSKGDFIV 178
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                ||::| |  ||:||::||  ||:|:  | :||  |:  :|:|: ||| | |    |   :
Db        121 NRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKANFKIRHNI--EDGSVQL 178

Qy        179 ADHTQMNTPIGGGPVHVPEYHHMSYHVKLSKDVTDHRDNMSLKETVRA 226
              ||| | ||||| ||| :|: |::|    ||||  : ||:| | | | |
Db        179 ADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTA 226


RESULT 6
US-11-149-177-6 (SEQ ID No. 6)
; Sequence 6, Application US/11149177
; Publication No. US20080213879A1
; GENERAL INFORMATION
;  APPLICANT: BAUBET, VALERIE
;  APPLICANT:LE MOUELLIC, HERVE
;  APPLICANT:BRULET, PHILIPPE
;  TITLE OF INVENTION: CHIMERIC GFP-AEQUORIN AS BIOLUMINESCENT Ca++ REPORTERS
;  TITLE OF INVENTION:AT THE SINGLE CELL LEVEL
;  FILE REFERENCE: 03495-0207-00000
;  CURRENT APPLICATION NUMBER: US/11/149,177
;  CURRENT FILING DATE: 2005-06-10
;  PRIOR APPLICATION NUMBER: 09863901
;  PRIOR FILING DATE: 2001-05-24
;  PRIOR APPLICATION NUMBER: 60/208,314
;  PRIOR FILING DATE: 2000-06-01
;  PRIOR APPLICATION NUMBER: 60/210,526
;  PRIOR FILING DATE: 2000-06-06
;  PRIOR APPLICATION NUMBER: 60/255,111
;  PRIOR FILING DATE: 2000-12-14
;  NUMBER OF SEQ ID NOS: 48
;  SOFTWARE: PatentIn Ver. 2.1
; SEQ ID NO 6
;  LENGTH: 906
;  TYPE: PRT
;  ORGANISM: Aequorea victoria
US-11-149-177-6

  Query Match             50.5%;  Score 648;  DB 4;  Length 906;
  Best Local Similarity   53.9%;  Pred. No. 2e-59;
  Matches  123;  Conservative   40;  Mismatches   61;  Indels    4;  Gaps    2;

Qy          1 MSSGALLFHGKIPYVVEMEGNVDGHTFSIRGKGYGDASVGKVDAQFICTTGDVPVPWSTL 60
              || |  || | :| :||::|:|:|| ||: |:| |||: ||:  :|||||| :|||| ||
Db        430 MSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTL 489

Qy         61 VTTLTYGAQCFAKYGPELK--DFYKSCMPDGYVQERTITFEGDGNFKTRAEVTFENGSVY 118
              ||||||| |||::|   :|  ||:|| ||:|||||||| |: |||:|||||| ||  ::
Db        490 VTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLV 549

Qy        119 NRVKLNGQGFKKDGHVLGKNLEFNFTPHCLYIWGDQANHGLKSAFKICHEITGSKGDFIV 178
              ||::| |  ||:||::||  ||:|:  | :||  |:  :|:|: ||| | |    |   :
Db        550 NRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKANFKIRHNI--EDGSVQL 607

Qy        179 ADHTQMNTPIGGGPVHVPEYHHMSYHVKLSKDVTDHRDNMSLKETVRA 226
              ||| | ||||| ||| :|: |::|    ||||  : ||:| | | | |
Db        608 ADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTA 655
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(B) Alignment of SEQ ID No. 9 of instant application with SEQ ID numbers 7-12 of Baubet et al.

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RESULT 1
US-11-149-177-9 (SEQ ID No. 9)
; Sequence 9, Application US/11149177
; Publication No. US20080213879A1
; GENERAL INFORMATION
;  APPLICANT: BAUBET, VALERIE
;  APPLICANT:LE MOUELLIC, HERVE
;  APPLICANT:BRULET, PHILIPPE
;  TITLE OF INVENTION: CHIMERIC GFP-AEQUORIN AS BIOLUMINESCENT Ca++ REPORTERS
;  TITLE OF INVENTION:AT THE SINGLE CELL LEVEL
;  FILE REFERENCE: 03495-0207-00000
;  CURRENT APPLICATION NUMBER: US/11/149,177
;  CURRENT FILING DATE: 2005-06-10
;  PRIOR APPLICATION NUMBER: 09863901
;  PRIOR FILING DATE: 2001-05-24
;  PRIOR APPLICATION NUMBER: 60/208,314
```

```
;  PRIOR FILING DATE: 2000-06-01
;  PRIOR APPLICATION NUMBER: 60/210,526
;  PRIOR FILING DATE: 2000-06-06
;  PRIOR APPLICATION NUMBER: 60/255,111
;  PRIOR FILING DATE: 2000-12-14
;  NUMBER OF SEQ ID NOS: 48
;  SOFTWARE: PatentIn Ver. 2.1
; SEQ ID NO 9
;  LENGTH: 1350
;  TYPE: DNA
;  ORGANISM: Aequorea victoria
US-11-149-177-9

  Query Match             47.1%;  Score 332.2;  DB 3;  Length 1350;
  Best Local Similarity   70.1%;  Pred. No. 1.2e-73;
  Matches  480;  Conservative    0;  Mismatches  193;  Indels   12;  Gaps    2;

Qy          1 ATGAGCAGCGGCGCCCTGCTGTTCCACGGCAAGATCCCCTACGTGGTGGAGATGGAGGGC 60
              |||||||  ||||    ||||||  |||  | | |||  | |||| ||| |||| |||
Db          1 ATGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGC 60

Qy         61 AATGTGGATGGCCACACCTTCAGCATCCGCGGCAAGGGCTACGGCGATGCCAGCGTGGGC 120
               | ||  | |||||||  ||||||| |   |||| ||||| | |||||||||| |   |||
Db         61 GACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGC 120

Qy        121 AAGGTGGATGCCCAGTTCATCTGCACCACCGGCGATGTGCCCGTGCCCTGGAGCACCCTG 180
              ||| ||       |||||||||||||||||||| |  |||||||||||||  ||||||
Db        121 AAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTC 180

Qy        181 GTGACCACCCTGACCTACGGCGCCCAGTGCTTCGCCAAGTACGGCCCCGAGCTGAAG--- 237
              ||||||||||||||||||||||  |||||||||||  |   |||  |  | |  |||||
Db        181 GTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAG 240

Qy        238 ---GATTTCTACAAGAGCTGCATGCCCGATGGCTACGTGCAGGAGCGCACCATCACCTTC 294
                 || ||||| ||||  |  ||||||||| |||||||| ||||||||||||||||  ||||
Db        241 CACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTC 300

Qy        295 GAGGGCGATGGCAATTTCAAGACCCGCGCCGAGGTGACCTTCGAGAATGGCAGCGTGTAC 354
               ||| ||| ||||| | |||||||||||||||||||  ||||||   | || | ||
Db        301 AAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTG 360

Qy        355 AATCGCGTGAAGCTGAATGGCCAGGGCTTCAAGAAGGATGGCCACGTGCTGGGCAAGAAT 414
              || ||| |  ||||||| ||||   | |||||| |||| ||| || | ||||||  | ||
Db        361 AACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAG 420

Qy        415 CTGGAGTTCAATTTCACCCCCCACTGCCTGTACATCTGGGGCGATCAGGCCAATCACGGC 474
              ||||||| ||| | || |  ||||  | | || |||  || |||  ||   ||  |||||
Db        421 CTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGC 480

Qy        475 CTGAAGAGCGCCTTCAAGATCTGCCACGAGATCACCGGCAGCAAGGGCGATTTCATCGTG 534
               | |||  |  ||||||||||| ||||| | |||   | | |||  |   |  ||
Db        481 ATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTC------ 534

Qy        535 GCCGATCACACCCAGATGAATACCCCCATCGGCGGCGGCCCCGTGCACGTGCCCGAGTAC 594
              ||||| |||  ||||  |||  ||||||||||||| |||||||||||   |||||||  ||
Db        535 GCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAAC 594

Qy        595 CACCACATGAGCTACCACGTGAAGCTGAGCAAGGATGTGACCGATCACCGCGATAATATG 654
              ||| || |||||  |||       |||||||| ||    | |||  | |||||| | |||
Db        595 CACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATG 654

Qy        655 AGCCTGAAGGAGACCGTGCGCGCCG 679
                ||||  ||||  ||||  |||||
Db        655 GTCCTGCTGGAGTTCGTGACCGCCG 679


RESULT 2
US-11-149-177-10 (SEQ ID No. 10)
; Sequence 10, Application US/11149177
; Publication No. US20080213879A1
; GENERAL INFORMATION
;  APPLICANT: BAUBET, VALERIE
;  APPLICANT:LE MOUELLIC, HERVE
;  APPLICANT:BRULET, PHILIPPE
;  TITLE OF INVENTION: CHIMERIC GFP-AEQUORIN AS BIOLUMINESCENT Ca++ REPORTERS
;  TITLE OF INVENTION:AT THE SINGLE CELL LEVEL
;  FILE REFERENCE: 03495-0207-00000
;  CURRENT APPLICATION NUMBER: US/11/149,177
;  CURRENT FILING DATE: 2005-06-10
;  PRIOR APPLICATION NUMBER: 09863901
;  PRIOR FILING DATE: 2001-05-24
;  PRIOR APPLICATION NUMBER: 60/208,314
;  PRIOR FILING DATE: 2000-06-01
```

```
;  PRIOR APPLICATION NUMBER: 60/210,526
;  PRIOR FILING DATE: 2000-06-06
;  PRIOR APPLICATION NUMBER: 60/255,111
;  PRIOR FILING DATE: 2000-12-14
;  NUMBER OF SEQ ID NOS: 48
;  SOFTWARE: PatentIn Ver. 2.1
; SEQ ID NO 10
;  LENGTH: 1404
;  TYPE: DNA
;  ORGANISM: Aequorea victoria
US-11-149-177-10

  Query Match             47.1%;  Score 332.2;  DB 3;  Length 1404;
  Best Local Similarity   70.1%;  Pred. No. 1.2e-73;
  Matches  480;  Conservative    0;  Mismatches  193;  Indels   12;  Gaps    2;

Qy             1 ATGAGCAGCGGCGCCCTGCTGTTCCACGGCAAGATCCCCTACGTGGTGGAGATGGAGGGC 60
                 |||||||  ||||    |||||||  |||   | | |||  | |||| ||| |||| |||
Db             1 ATGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGC 60

Qy            61 AATGTGGATGGCCACACCTTCAGCATCCGCGGCAAGGGCTACGGCGATGCCAGCGTGGGC 120
                  | ||  | |||||||  ||||||| |   |||| ||||| | |||||||||| |   |||
Db            61 GACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGC 120

Qy           121 AAGGTGGATGCCCAGTTCATCTGCACCACCGGCGATGTGCCCGTGCCCTGGAGCACCCTG 180
                 ||| ||       ||||||||||||||||||||| |  ||||||||||||||  |||||| 
Db           121 AAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTC 180

Qy           181 GTGACCACCCTGACCTACGGCGCCCAGTGCTTCGCCAAGTACGGCCCCGAGCTGAAG--- 237
                 ||||||||||||||||||||||  |||||||||  |   |||  |  | |  |||||   
Db           181 GTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAG 240

Qy           238 ---GATTTCTACAAGAGCTGCATGCCCGATGGCTACGTGCAGGAGCGCACCATCACCTTC 294
                    || |||| ||||  |  ||||||||| |||||||| ||||||||||||||  ||||
Db           241 CACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTC 300

Qy           295 GAGGGCGATGGCAATTTCAAGACCCGCGCCGAGGTGACCTTCGAGAATGGCAGCGTGTAC 354
                  ||| ||| ||||| | |||||||||||||||||||  ||||||   | || | ||   
Db           301 AAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTG 360

Qy           355 AATCGCGTGAAGCTGAATGGCCAGGGCTTCAAGAAGGATGGCCACGTGCTGGGCAAGAAT 414
                 || ||| |  ||||||| ||| |   | |||||||| |||| ||| || | |||||  | || 
Db           361 AACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAG 420

Qy           415 CTGGAGTTCAATTTCACCCCCCACTGCCTGTACATCTGGGGCGATCAGGCCAATCACGGC 474
                 ||||||| ||| | || |  ||||  |   || |||  || |||  ||   ||  |||||
Db           421 CTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGC 480

Qy           475 CTGAAGAGCGCCTTCAAGATCTGCCACGAGATCACCGGCAGCAAGGGCGATTTCATCGTG 534
                  | |||  |  ||||||||||| ||||| | |||   | | |||  |   |  ||      
Db           481 ATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTC------ 534

Qy           535 GCCGATCACACCCAGATGAATACCCCCATCGGCGGCGGCCCCGTGCACGTGCCCGAGTAC 594
                 ||||| |||  ||||  ||| ||||||||||||| ||||||||||   |||||||  ||
Db           535 GCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAAC 594

Qy           595 CACCACATGAGCTACCACGTGAAGCTGAGCAAGGATGTGACCGATCACCGCGATAATATG 654
                 ||| || |||||  |||       |||||||| ||    | |||  | |||||| | |||
Db           595 CACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATG 654

Qy           655 AGCCTGAAGGAGACCGTGCGCGCCG 679
                   ||||  ||||  ||||  |||||
Db           655 GTCCTGCTGGAGTTCGTGACCGCCG 679


RESULT 3
US-11-149-177-11 (SEQ ID No. 11)
; Sequence 11, Application US/11149177
; Publication No. US20080213879A1
; GENERAL INFORMATION
;  APPLICANT: BAUBET, VALERIE
;  APPLICANT:LE MOUELLIC, HERVE
;  APPLICANT:BRULET, PHILIPPE
;  TITLE OF INVENTION: CHIMERIC GFP-AEQUORIN AS BIOLUMINESCENT Ca++ REPORTERS
;  TITLE OF INVENTION:AT THE SINGLE CELL LEVEL
;  FILE REFERENCE: 03495-0207-00000
;  CURRENT APPLICATION NUMBER: US/11/149,177
;  CURRENT FILING DATE: 2005-06-10
;  PRIOR APPLICATION NUMBER: 09863901
;  PRIOR FILING DATE: 2001-05-24
;  PRIOR APPLICATION NUMBER: 60/208,314
;  PRIOR FILING DATE: 2000-06-01
;  PRIOR APPLICATION NUMBER: 60/210,526
```

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;  PRIOR FILING DATE: 2000-06-06
;  PRIOR APPLICATION NUMBER: 60/255,111
;  PRIOR FILING DATE: 2000-12-14
;  NUMBER OF SEQ ID NOS: 48
;  SOFTWARE: PatentIn Ver. 2.1
; SEQ ID NO 11
;  LENGTH: 1431
;  TYPE: DNA
;  ORGANISM: Aequorea victoria
US-11-149-177-11

  Query Match             47.1%;  Score 332.2;  DB 3;  Length 1431;
  Best Local Similarity   70.1%;  Pred. No. 1.2e-73;
  Matches  480;  Conservative    0;  Mismatches  193;  Indels   12;  Gaps    2;

Qy          1 ATGAGCAGCGGCGCCCTGCTGTTCCACGGCAAGATCCCCTACGTGGTGGAGATGGAGGGC 60
              |||||||  ||||    |||||||  |||   | | |||  | |||| ||| |||| |||
Db          1 ATGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGC 60

Qy         61 AATGTGGATGGCCACACCTTCAGCATCCGCGGCAAGGGCTACGGCGATGCCAGCGTGGGC 120
               | ||  | |||||||  |||||| |   |||| ||||| | |||||||||| |   |||
Db         61 GACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGC 120

Qy        121 AAGGTGGATGCCCAGTTCATCTGCACCACCGGCGATGTGCCCGTGCCCTGGAGCACCCTG 180
              ||| ||       |||||||||||||||||||| |  ||||||||||||||  ||||||
Db        121 AAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTC 180

Qy        181 GTGACCACCCTGACCTACGGCGCCCAGTGCTTCGCCAAGTACGGCCCCGAGCTGAAG--- 237
              ||||||||||||||||||||||  |||||||||  |   |||  |  | |  |||||
Db        181 GTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAG 240

Qy        238 ---GATTTCTACAAGAGCTGCATGCCCGATGGCTACGTGCAGGAGCGCACCATCACCTTC 294
                 || |||| ||||  |  ||||||||| |||||||| |||||||||||||||  ||||
Db        241 CACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTC 300

Qy        295 GAGGGCGATGGCAATTTCAAGACCCGCGCCGAGGTGACCTTCGAGAATGGCAGCGTGTAC 354
               ||| ||| ||||| | ||||||||||||||||||||  ||||||   | || | ||
Db        301 AAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTG 360

Qy        355 AATCGCGTGAAGCTGAATGGCCAGGGCTTCAAGAAGGATGGCCACGTGCTGGGCAAGAAT 414
              || ||| |  ||||||| |||   | ||||||| |||| ||| || | |||||  | ||
Db        361 AACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAG 420

Qy        415 CTGGAGTTCAATTTCACCCCCCACTGCCTGTACATCTGGGGCGATCAGGCCAATCACGGC 474
              ||||||| ||| | || |  ||||  | | || |||  || |||  ||   ||  |||||
Db        421 CTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGC 480

Qy        475 CTGAAGAGCGCCTTCAAGATCTGCCACGAGATCACCGGCAGCAAGGGCGATTTCATCGTG 534
               | |||  |  |||||||||| ||||| | |||   | | |||  |   |  ||
Db        481 ATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTC------ 534

Qy        535 GCCGATCACACCCAGATGAATACCCCCATCGGCGGCGGCCCCGTGCACGTGCCCGAGTAC 594
              ||||| |||  ||||  ||| ||||||||||||| |||||||||||   |||||||  ||
Db        535 GCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAAC 594

Qy        595 CACCACATGAGCTACCACGTGAAGCTGAGCAAGGATGTGACCGATCACCGCGATAATATG 654
              ||| || |||||  |||       |||||||| ||    | |||  | |||||| | |||
Db        595 CACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATG 654

Qy        655 AGCCTGAAGGAGACCGTGCGCGCCG 679
                ||||  ||||  ||||  |||||
Db        655 GTCCTGCTGGAGTTCGTGACCGCCG 679


RESULT 4
US-11-149-177-8 (SEQ ID No. 8)
; Sequence 8, Application US/11149177
; Publication No. US20080213879A1
; GENERAL INFORMATION
;  APPLICANT: BAUBET, VALERIE
;  APPLICANT:LE MOUELLIC, HERVE
;  APPLICANT:BRULET, PHILIPPE
;  TITLE OF INVENTION: CHIMERIC GFP-AEQUORIN AS BIOLUMINESCENT Ca++ REPORTERS
;  TITLE OF INVENTION:AT THE SINGLE CELL LEVEL
;  FILE REFERENCE: 03495-0207-00000
;  CURRENT APPLICATION NUMBER: US/11/149,177
;  CURRENT FILING DATE: 2005-06-10
;  PRIOR APPLICATION NUMBER: 09863901
;  PRIOR FILING DATE: 2001-05-24
;  PRIOR APPLICATION NUMBER: 60/208,314
;  PRIOR FILING DATE: 2000-06-01
;  PRIOR APPLICATION NUMBER: 60/210,526
;  PRIOR FILING DATE: 2000-06-06
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;  PRIOR APPLICATION NUMBER: 60/255,111
;  PRIOR FILING DATE: 2000-12-14
;  NUMBER OF SEQ ID NOS: 48
;  SOFTWARE: PatentIn Ver. 2.1
; SEQ ID NO 8
;  LENGTH: 2673
;  TYPE: DNA
;  ORGANISM: Aequorea victoria
US-11-149-177-8

  Query Match             47.1%;  Score 332.2;  DB 3;  Length 2673;
  Best Local Similarity   70.1%;  Pred. No. 1.3e-73;
  Matches  480;  Conservative    0;  Mismatches  193;  Indels   12;  Gaps    2;

Qy          1 ATGAGCAGCGGCGCCCTGCTGTTCCACGGCAAGATCCCCTACGTGGTGGAGATGGAGGGC 60
              |||||||  ||||    |||||||  |||   | | |||  | |||| ||| |||| |||
Db          1 ATGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGC 60

Qy         61 AATGTGGATGGCCACACCTTCAGCATCCGCGGCAAGGGCTACGGCGATGCCAGCGTGGGC 120
               | ||  | |||||||  |||||| |   |||| ||||| | |||||||||| |   |||
Db         61 GACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGC 120

Qy        121 AAGGTGGATGCCCAGTTCATCTGCACCACCGGCGATGTGCCCGTGCCCTGGAGCACCCTG 180
              ||| ||       |||||||||||||||||||| |  ||||||||||||||  ||||||
Db        121 AAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTC 180

Qy        181 GTGACCACCCTGACCTACGGCGCCCAGTGCTTCGCCAAGTACGGCCCCGAGCTGAAG--- 237
              ||||||||||||||||||||||  |||||||||  |   |||  |  | |  |||||
Db        181 GTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAG 240

Qy        238 ---GATTTCTACAAGAGCTGCATGCCCGATGGCTACGTGCAGGAGCGCACCATCACCTTC 294
                 || |||| ||||  |  ||||||||| |||||||| |||||||||||||||  ||||
Db        241 CACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTC 300

Qy        295 GAGGGCGATGGCAATTTCAAGACCCGCGCCGAGGTGACCTTCGAGAATGGCAGCGTGTAC 354
               ||| ||| ||||| | ||||||||||||||||||||  ||||||   | || | ||
Db        301 AAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTG 360

Qy        355 AATCGCGTGAAGCTGAATGGCCAGGGCTTCAAGAAGGATGGCCACGTGCTGGGCAAGAAT 414
              || ||| |  ||||||| |||   | ||||||| |||| ||| || | |||||  | ||
Db        361 AACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAG 420

Qy        415 CTGGAGTTCAATTTCACCCCCCACTGCCTGTACATCTGGGGCGATCAGGCCAATCACGGC 474
              ||||||| ||| | || |  ||||  | | || |||  || |||  ||   ||  |||||
Db        421 CTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGC 480

Qy        475 CTGAAGAGCGCCTTCAAGATCTGCCACGAGATCACCGGCAGCAAGGGCGATTTCATCGTG 534
               | |||  |  |||||||||| ||||| | |||   | | |||  |   |  ||
Db        481 ATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTC------ 534

Qy        535 GCCGATCACACCCAGATGAATACCCCCATCGGCGGCGGCCCCGTGCACGTGCCCGAGTAC 594
              ||||| |||  ||||  ||| ||||||||||||| |||||||||||   |||||||  ||
Db        535 GCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAAC 594

Qy        595 CACCACATGAGCTACCACGTGAAGCTGAGCAAGGATGTGACCGATCACCGCGATAATATG 654
              ||| || |||||  |||       |||||||| ||    | |||  | |||||| | |||
Db        595 CACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATG 654

Qy        655 AGCCTGAAGGAGACCGTGCGCGCCG 679
                ||||  ||||  ||||  |||||
Db        655 GTCCTGCTGGAGTTCGTGACCGCCG 679
```

RESULT 5

US-11-149-177-12 **(SEQ ID No. 12)**

```
; Sequence 12, Application US/11149177
; Publication No. US20080213879A1
; GENERAL INFORMATION
;  APPLICANT: BAUBET, VALERIE
;  APPLICANT:LE MOUELLIC, HERVE
;  APPLICANT:BRULET, PHILIPPE
;  TITLE OF INVENTION: CHIMERIC GFP-AEQUORIN AS BIOLUMINESCENT Ca++ REPORTERS
;  TITLE OF INVENTION:AT THE SINGLE CELL LEVEL
;  FILE REFERENCE: 03495-0207-00000
;  CURRENT APPLICATION NUMBER: US/11/149,177
;  CURRENT FILING DATE: 2005-06-10
;  PRIOR APPLICATION NUMBER: 09863901
;  PRIOR FILING DATE: 2001-05-24
;  PRIOR APPLICATION NUMBER: 60/208,314
;  PRIOR FILING DATE: 2000-06-01
;  PRIOR APPLICATION NUMBER: 60/210,526
;  PRIOR FILING DATE: 2000-06-06
;  PRIOR APPLICATION NUMBER: 60/255,111
```

```
;  PRIOR FILING DATE: 2000-12-14
;  NUMBER OF SEQ ID NOS: 48
;  SOFTWARE: PatentIn Ver. 2.1
; SEQ ID NO 12
;  LENGTH: 2718
;  TYPE: DNA
;  ORGANISM: Aequorea victoria
US-11-149-177-12

  Query Match             47.1%;  Score 332.2;  DB 3;  Length 2718;
  Best Local Similarity   70.1%;  Pred. No. 1.3e-73;
  Matches  480;  Conservative    0;  Mismatches  193;  Indels   12;  Gaps    2;

Qy          1 ATGAGCAGCGGCGCCCTGCTGTTCCACGGCAAGATCCCCTACGTGGTGGAGATGGAGGGC 60
              |||||||  ||||    |||||||  |||   | | |||  | |||| ||| |||| |||
Db       1288 ATGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGC 1347

Qy         61 AATGTGGATGGCCACACCTTCAGCATCCGCGGCAAGGGCTACGGCGATGCCAGCGTGGGC 120
               | ||  | |||||||  |||||| |   |||| ||||| | |||||||||| |   |||
Db       1348 GACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGC 1407

Qy        121 AAGGTGGATGCCCAGTTCATCTGCACCACCGGCGATGTGCCCGTGCCCTGGAGCACCCTG 180
              ||| ||       |||||||||||||||||||| |  ||||||||||||||  ||||||
Db       1408 AAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTC 1467

Qy        181 GTGACCACCCTGACCTACGGCGCCCAGTGCTTCGCCAAGTACGGCCCCGAGCTGAAG--- 237
              ||||||||||||||||||||||  |||||||||  |   |||  |  | |  |||||
Db       1468 GTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAG 1527

Qy        238 ---GATTTCTACAAGAGCTGCATGCCCGATGGCTACGTGCAGGAGCGCACCATCACCTTC 294
                 || |||| ||||  |  ||||||||| |||||||| |||||||||||||||  ||||
Db       1528 CACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTC 1587

Qy        295 GAGGGCGATGGCAATTTCAAGACCCGCGCCGAGGTGACCTTCGAGAATGGCAGCGTGTAC 354
               ||| ||| ||||| | ||||||||||||||||||||  ||||||   | || | ||
Db       1588 AAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTG 1647

Qy        355 AATCGCGTGAAGCTGAATGGCCAGGGCTTCAAGAAGGATGGCCACGTGCTGGGCAAGAAT 414
              || ||| |  ||||||| |||   | ||||||| |||| ||| || | |||||  | ||
Db       1648 AACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAG 1707

Qy        415 CTGGAGTTCAATTTCACCCCCCACTGCCTGTACATCTGGGGCGATCAGGCCAATCACGGC 474
              ||||||| ||| | || |  ||||  | | || |||  || |||  ||   ||  |||||
Db       1708 CTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGC 1767

Qy        475 CTGAAGAGCGCCTTCAAGATCTGCCACGAGATCACCGGCAGCAAGGGCGATTTCATCGTG 534
               | |||  |  |||||||||| ||||| | |||   | | |||  |   |  ||
Db       1768 ATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTC------ 1821

Qy        535 GCCGATCACACCCAGATGAATACCCCCATCGGCGGCGGCCCCGTGCACGTGCCCGAGTAC 594
              ||||| |||  ||||  ||| ||||||||||||| |||||||||||   |||||||  ||
Db       1822 GCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAAC 1881

Qy        595 CACCACATGAGCTACCACGTGAAGCTGAGCAAGGATGTGACCGATCACCGCGATAATATG 654
              ||| || |||||  |||       |||||||| ||    | |||  | |||||| | |||
Db       1882 CACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATG 1941

Qy        655 AGCCTGAAGGAGACCGTGCGCGCCG 679
                ||||  ||||  ||||  |||||
Db       1942 GTCCTGCTGGAGTTCGTGACCGCCG 1966
```

RESULT 6
US-11-149-177-7 **(SEQ ID No. 7)**

```
; Sequence 7, Application US/11149177
; Publication No. US20080213879A1
; GENERAL INFORMATION
;  APPLICANT: BAUBET, VALERIE
;  APPLICANT:LE MOUELLIC, HERVE
;  APPLICANT:BRULET, PHILIPPE
;  TITLE OF INVENTION: CHIMERIC GFP-AEQUORIN AS BIOLUMINESCENT Ca++ REPORTERS
;  TITLE OF INVENTION:AT THE SINGLE CELL LEVEL
;  FILE REFERENCE: 03495-0207-00000
;  CURRENT APPLICATION NUMBER: US/11/149,177
;  CURRENT FILING DATE: 2005-06-10
;  PRIOR APPLICATION NUMBER: 09863901
;  PRIOR FILING DATE: 2001-05-24
;  PRIOR APPLICATION NUMBER: 60/208,314
;  PRIOR FILING DATE: 2000-06-01
;  PRIOR APPLICATION NUMBER: 60/210,526
;  PRIOR FILING DATE: 2000-06-06
;  PRIOR APPLICATION NUMBER: 60/255,111
;  PRIOR FILING DATE: 2000-12-14
```

```
;  NUMBER OF SEQ ID NOS: 48
;  SOFTWARE: PatentIn Ver. 2.1
; SEQ ID NO 7
;  LENGTH: 3973
;  TYPE: DNA
;  ORGANISM: Aequorea victoria
US-11-149-177-7

  Query Match             47.1%;  Score 332.2;  DB 3;  Length 3973;
  Best Local Similarity   70.1%;  Pred. No. 1.3e-73;
  Matches  480;  Conservative    0;  Mismatches  193;  Indels   12;  Gaps    2;

Qy          1 ATGAGCAGCGGCGCCCTGCTGTTCCACGGCAAGATCCCCTACGTGGTGGAGATGGAGGGC 60
              |||||||  ||||    |||||||  |||   | | |||  | |||| ||| |||| |||
Db          1 ATGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGC 60

Qy         61 AATGTGGATGGCCACACCTTCAGCATCCGCGGCAAGGGCTACGGCGATGCCAGCGTGGGC 120
               | ||  | |||||||  |||||| |   |||| ||||| | |||||||||| |   |||
Db         61 GACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGC 120

Qy        121 AAGGTGGATGCCCAGTTCATCTGCACCACCGGCGATGTGCCCGTGCCCTGGAGCACCCTG 180
              ||| ||       |||||||||||||||||||| |  ||||||||||||||  ||||||
Db        121 AAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTC 180

Qy        181 GTGACCACCCTGACCTACGGCGCCCAGTGCTTCGCCAAGTACGGCCCCGAGCTGAAG--- 237
              ||||||||||||||||||||||  |||||||||  |   |||  |  | |  |||||
Db        181 GTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAG 240

Qy        238 ---GATTTCTACAAGAGCTGCATGCCCGATGGCTACGTGCAGGAGCGCACCATCACCTTC 294
                 || |||| ||||  |  ||||||||| |||||||| |||||||||||||||  ||||
Db        241 CACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTC 300

Qy        295 GAGGGCGATGGCAATTTCAAGACCCGCGCCGAGGTGACCTTCGAGAATGGCAGCGTGTAC 354
               ||| ||| ||||| | ||||||||||||||||||||  ||||||   | || | ||
Db        301 AAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTG 360

Qy        355 AATCGCGTGAAGCTGAATGGCCAGGGCTTCAAGAAGGATGGCCACGTGCTGGGCAAGAAT 414
              || ||| |  ||||||| |||   | ||||||| |||| ||| || | |||||  | ||
Db        361 AACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAG 420

Qy        415 CTGGAGTTCAATTTCACCCCCCACTGCCTGTACATCTGGGGCGATCAGGCCAATCACGGC 474
              ||||||| ||| | || |  ||||  | | || |||  || |||  ||   ||  |||||
Db        421 CTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGC 480

Qy        475 CTGAAGAGCGCCTTCAAGATCTGCCACGAGATCACCGGCAGCAAGGGCGATTTCATCGTG 534
               | |||  |  |||||||||| ||||| | |||   | | |||  |   |  ||
Db        481 ATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTC------ 534

Qy        535 GCCGATCACACCCAGATGAATACCCCCATCGGCGGCGGCCCCGTGCACGTGCCCGAGTAC 594
              ||||| |||  ||||  ||| ||||||||||||| |||||||||||   |||||||  ||
Db        535 GCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAAC 594

Qy        595 CACCACATGAGCTACCACGTGAAGCTGAGCAAGGATGTGACCGATCACCGCGATAATATG 654
              ||| || |||||  |||       |||||||| ||    | |||  | |||||| | |||
Db        595 CACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATG 654

Qy        655 AGCCTGAAGGAGACCGTGCGCGCCG 679
                ||||  ||||  ||||  |||||
Db        655 GTCCTGCTGGAGTTCGTGACCGCCG 679
```

Thus, Baubet et al. (US 2008/0213879) clearly anticipates claims 1, 5-8, 13, 17, and 27-33 of instant application.

Applicant's arguments

Applicant argues that amended claim 1 recites that the sequences have at least 85% identity with full length SEQ ID NO:10, and that, as such, Baubet et al. do not teach this claim

element. Accordingly, Baubet et al. do not anticipate claim 1 or its dependents (See page 18 of Applicant's remarks filed on 05/14/2009).

Applicant argues that amended claim 13 recites "A nucleic acid molecule having a sequence that is substantially the same as, or identical to a nucleotide sequence of at least 300 nucleotides in length of the nucleic acid molecule according to claim 1." Thus, what is claimed is a nucleic acid molecule having a sequence that is substantially the same as, or identical to, a nucleotide sequence of at least 300 nucleotides in length of a nucleic acid molecule that encodes a protein having 85% identity with SEQ ID NO:10. The Applicants submit that for such to be the case, the nucleic acid molecule of claim 13 must have its nucleotides in the same sequence as the nucleic acid molecule of claim 1. Accordingly, Applicant argues that, contrary to the Examiner's assertions, the claim does not read on identical sequences that are not necessarily continuous. Furthermore, the Applicants submit that Baubet et al. do not teach this claim element because Baubet et al. teaches nucleic acid sequence that has, at most, 47.1% identity with a nucleotide sequence of at least 300 contiguous nucleotides in length that encode a protein having at least 85% identity with full length SEQ ID NO: 10; see, for example, the alignments provided by the Examiner, pages 20-26 of the Office Action. As such, Baubet does not teach pending Claim 13, and thus does not anticipate the pending claims (See page 190 of Applicant's remarks filed on 05/14/2009).

Response to Applicant's arguments

As Stated in this maintained rejection, the following claim interpretations are applied in this rejection.

(i) Amended claim 13 filed on 05/14/2009 reads as follows: A nucleic acid molecule having a sequence that is substantially the same as, or identical to a nucleotide sequence of at least 300 nucleotides in length of the nucleic acid molecule according to claim 1. The limitation "at least 300 nucleotides in length of the nucleic acid molecule" reads on those identical nucleotide sequences that are not necessarily continuous. Accordingly, this limitation requires 100 amino acid residues (which correspond to 300 nucleotides) identical to SEQ ID No: 10 (full length 234 amino acid residues). In other words, this limitation requires at least 42.7% (100/234=42.7%) identical to full length SEQ ID No: 10.

It is noted that the sequences of SEQ ID numbers 1-6 taught by Baubet et al. are 50.5% identical to the full length of SEQ ID No: 10 of instant application. It is worth noting that claim 13 as amended is not further limiting claim 1. In fact, claim 13 as amended is further broadening the scope of claim 1.

(ii) Amended claim 1 filed on 05/14/2009 reads as follows: An isolated nucleic acid molecule encoding a fluorescent protein, wherein said protein has at least 85% identity with full length SEQ ID NO: 10. As discussed in the rejection of claims 1, 5-8, 13, 17, and 27-33 under 35 U.S.C 112 second and claim interpretation stated in **(i)**, the scope of claim 1 becomes unclear when the scope of claim 1 is narrower than the scope of its dependent claims 13 and 27-33. Accordingly, as the scope of claim 1 becomes unclear, dependent claims 5-8 and 17 become unclear. Therefore, considering the scope of dependent claims 13 and 27-33, the limitation "at least 85% identity with full length SEQ ID No: 10" recited in claim 1 is interpreted to encompass a fluorescent protein having a fragment with at least 9 out 10 amino acid residues identical to (i.e. 90% identity) the sequences of the full length SEQ ID NO: 10.

7. Claims 1, 5-8, 13, 17, and 27-30 remain rejected and newly added claims 31-33 are rejected under 35 U.S.C. 102(b) as being anticipated by Baubet et al. (PCT/EP01/07057, WO 2001/092300, filed on 06/01/2001). Applicant's arguments filed 05/14/2009 have been fully considered and they are not persuasive. Previous rejection is ***maintained*** for the reasons of record advanced on pages 26-28 of the office action mailed on 02/11/2009.

For the clarity and completeness of this office action, the rejection for the reasons of record advanced on pages 26-28 of the office action mailed on 02/11/2009, is reiterated below with revisions addressing claim amendments filed on 05/14/2009.

The following claim interpretations are applied in this rejection.

(i) Amended claim 13 filed on 05/14/2009 reads as follows: A nucleic acid molecule having a sequence that is substantially the same as, or identical to a nucleotide sequence of at least 300 nucleotides in length of the nucleic acid molecule according to claim 1. The limitation "at least 300 nucleotides in length of the nucleic acid molecule" reads on those identical nucleotide sequences that are not necessarily continuous. Accordingly, this limitation requires 100 amino acid residues (which correspond to 300 nucleotides) identical to SEQ ID No: 10 (full length 234 amino acid residues). In other words, this limitation requires at least 42.7% (100/234=42.7%) identical to full length SEQ ID No: 10.

(ii) Amended claim 1 filed on 05/14/2009 reads as follows: An isolated nucleic acid molecule encoding a fluorescent protein, wherein said protein has at least 85% identity with full length SEQ ID NO: 10. As discussed in the rejection of claims 1, 5-8, 13, 17, and 27-33 under 35 U.S.C 112 second and claim interpretation stated in **(i)**, the scope of claim 1 becomes unclear when the scope of claim 1 is narrower than the scope of its dependent claims 13 and 27-33. Accordingly, as the scope of claim 1 becomes unclear, dependent claims 5-8 and 17 become unclear. Therefore, considering the scope of dependent claims of claim 1, the limitation "at least 85% identity with full length SEQ ID No: 10" recited in claim 1 is interpreted to encompass a fluorescent protein having a fragment with at least 9 out 10 amino acid residues identical to (i.e. 90% identity) the sequences of the full length SEQ ID NO: 10.

With regard to claims 1, 5-8, 13, and 27-33, Baubet et al. teaches a modified bioluminescent system comprising a fluorescent molecule covalently linked with a photoprotein, wherein said link between the two proteins has the function to stabilize the modified bioluminescent system and allowing the transfer of the energy by Chemiluminescence Resonance Energy Transfer (CRET) in a host cell (See abstract and Figures 9-11, Baubet et al. US 2008/0213879). Baubet et al. teaches DNA construct with CMV promoter drive the expression of nucleic acid sequences encoding sequences of mutated GFP, followed by the

sequences of Poly A of SV40 (See Figure 1, PCT/EP01/07057, WO 2001/092300, filed on 06/01/2001).

With regard to the limitation "kit" recited in claim 17, Baubet et al. teaches kit for measuring the transfer of energy in vivo or in vitro contains at least one of the polypeptides according to the invention or the polynucleotide according to the invention and the reagents necessary for visualizing or detecting the said transfer in presence or in absence of a molecule of interest (See paragraph [0021], PCT/EP01/07057, WO 2001/092300, filed on 06/01/2001)

It is noted that Baubet et al. (PCT/EP01/07057, WO 2001/092300, filed on 06/01/2001) discloses the same DNA construct and SEQ ID Numbers as those disclosed in Baubet et al. (Baubet et al., US 2008/0213879). The sequence alignments have been presented in the preceding 102(e) rejection.

Thus, Baubet et al. (PCT/EP01/07057, WO 2001/092300, filed on 06/01/2001) clearly anticipates claims 1, 5-8, 13, 17, and 27-33 of instant application.

Applicant's arguments and Examiner's ***Response to Applicant's arguments*** are the same as documented in the maintained rejection of claims 1, 5-8, 13, 17, and 27-33 under 35 U.S.C. 102(e) as being anticipated by Baubet et al. (Baubet et al., US 2008/0213879, publication date 09/04/2008, Division of US 6,936,475, which is a Continuation of PCT/EP01/07057, WO 2001/092300, filed on 06/01/2001).

Conclusion

8. No claim is allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

Any inquiry concerning this communication from the examiner should be directed to Wu-Cheng Winston Shen whose telephone number is (571) 272-3157 and Fax number is 571-273-3157. The examiner can normally be reached on Monday through Friday from 8:00 AM to 4:30 PM. If attempts to reach the examiner by telephone are unsuccessful, the supervisory patent examiner, Peter Paras, Jr. can be reached on (571) 272-4517. The fax number for TC 1600 is (571) 273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Wu-Cheng Winston Shen/
Patent Examiner
Art Unit 1632